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# Solid Phase-Supported Synthesis of Muraymycin Analogues

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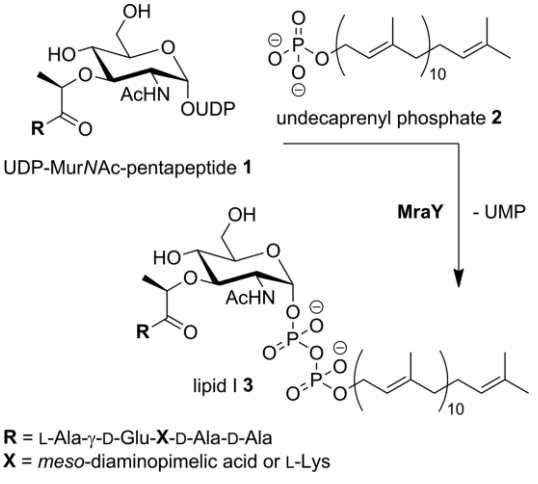
*Dedicated to Professor Armin de Meijere on the occasion of his 80th birthday.*

**Abstract:** Naturally occurring muraymycin nucleoside antibiotics represent a promising class of novel antimicrobials as they inhibit MraY, an enzyme involved in bacterial cell wall biosynthesis. The synthesis of muraymycins and their analogues is challenging as it involves multi-step routes, thus hampering detailed structure-activity relationship (SAR) studies. In this work, we report a novel solid phase-based synthetic strategy for accessing muraymycin analogues via a modular approach, thereby enabling a more efficient access to structural variations, particularly of the muraymycin peptide moiety. The efficiency of this new method was exemplified in an alanine scan of the peptide unit. The inhibitory in vitro activities of the resultant analogues towards MraY provided novel SAR insights. Overall, this new synthetic method for the preparation of muraymycin analogues might support the development of these antibacterial agents towards potential drug candidates.

## Introduction

Emerging bacteria with resistances against established antibiotics and therapeutic procedures represent a major issue in current and future healthcare.[1] Hence, the exploration of novel antibacterial targets and modes of action is required. One such promising target that is not addressed by currently used antibiotics is the membrane enzyme translocase I (MraY), which is involved in bacterial cell wall (peptidoglycan) biosynthesis.[2] Many antibiotics, e.g. the β-lactams, block the late extracellular steps of peptidoglycan formation.[3] However, the preceding intracellular steps (such as the MraY-mediated reaction) remain largely unexploited for antibacterial development, with the notable exception of the clinically used antibiotic fosfomycin inhibiting the enzyme MurA.

MraY catalyses the membrane-associated intracellular reaction of UDP-Mur*N*Ac-pentapeptide **1** with the membrane anchor undecaprenyl phosphate **2**, yielding lipid I **3** (Scheme 1).[4] The first X-ray crystal structure of this enzyme (from the extremophile *Aquifex aeolicus*) was published in 2013[5a] and largely confirmed previous in silico predictions on the transmembrane topology of MraY.[5b] The heterologous overexpression of MraY homologues from different bacteria has been reported, thus enabling more detailed studies on its biochemical properties.[6]




Scheme 1. MraY-catalysed reaction towards lipid I **3**.

A range of uridine-derived natural products ('nucleoside antibiotics') have been found to inhibit MraY, making them promising candidates for drug development efforts.[7] We focus our attention on the subclass of muraymycin nucleoside antibiotics.[7c] Muraymycins were first isolated in 2002 and demonstrated activity against the growth of several bacterial strains including *S. aureus*.[8] They are generally composed of a glycyluridine (GlyU) core unit linked (by a propyl linker) to a peptide moiety. One of the most active members of the subclass is muraymycin A1 **4** (Figure 1), bearing an aminoribose motif in the GlyU 5'-position. By now, even more active congeners have been identified.[8b]

In 2016, an X-ray co-crystal structure of MraY from *Aquifex aeolicus* in complex with muraymycin D2 **5** (Figure 1) as inhibitor was reported.[9] This work provided deeper insights into the binding mode of muraymycins to MraY, showing that the uridine motif and the 5'-aminoribose unit are bound in defined

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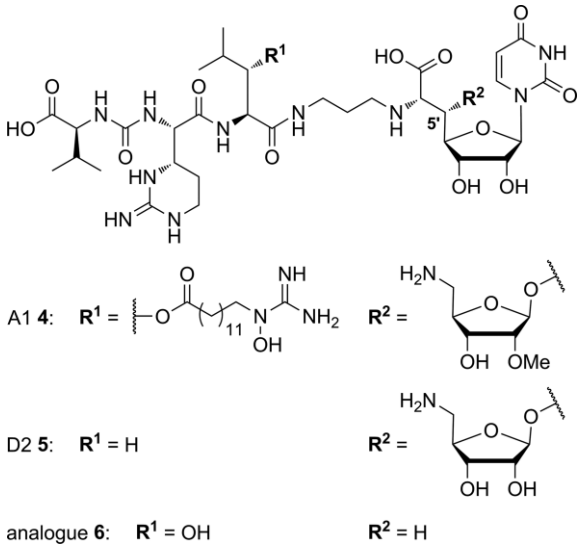
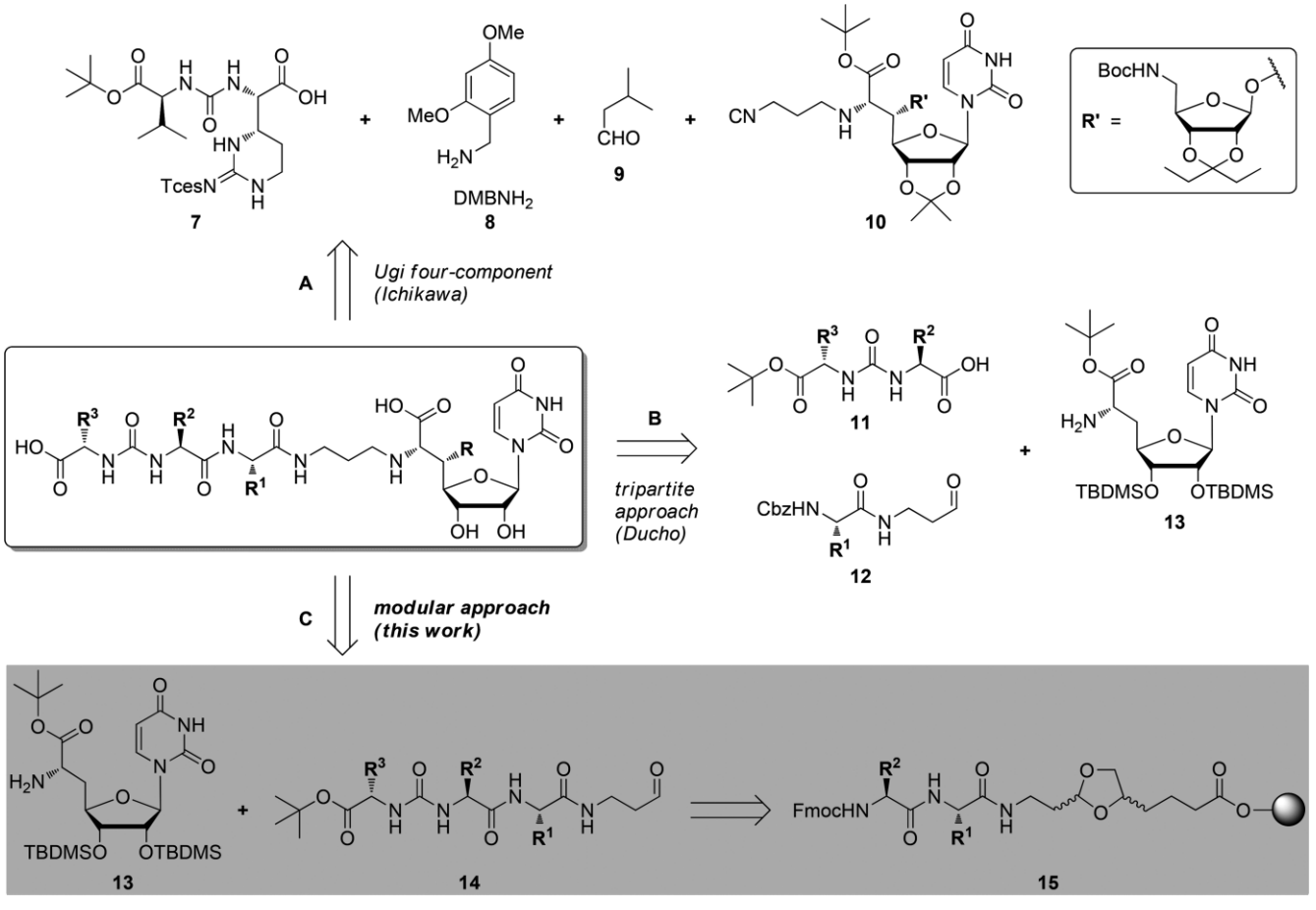



Figure 1. Structures of selected naturally occurring muraymycins **4** and **5** and of the bioactive synthetic 5′-defunctionalised analogue **6**.

pockets while the peptide moiety mainly lies on the surface of the protein. However, the significant conformational plasticity of the enzyme makes it difficult to predict a potential inhibitor's affinity by molecular modelling. Hence, experimental investigations on the structure-activity relationship (SAR) are the most promising option in order to develop muraymycins and their analogues towards antibacterial drug candidates. Several previous SAR studies on muraymycins and their analogues have demonstrated the relevance of specific parts of the muraymycin scaffold,[10] but variations in the peptide unit have not been comprehensively explored yet.

For further detailed SAR studies on muraymycins and their analogues (e.g. derivative **6**, Figure 1), an efficient synthetic access to these nucleoside-peptide hybrid structures is of utmost importance. Ichikawa et al. applied an Ugi four-component reaction to furnish muraymycins, e.g. muraymycin D2 **5** (Scheme 2, route A).[11] For this, they used building blocks **7**–**10** including uridine derivative **10**. The four-component reaction furnished a 1:1-mixture of epimers at the leucine moiety that could be separated by HPLC. As an alternative, we have developed a stereocontrolled tripartite approach (Scheme 2, route B) employing urea dipeptide **11**, aldehyde **12** and uridine derivative **13** as suitably protected building blocks.[10f] To reduce synthetic effort, we have mainly focussed on 5′-defunctionalised ('5′-deoxy') analogues[12] as some of these compounds (e.g. derivative **6**, Figure 1) were found to exhibit inhibitory activity towards MraY in the nanomolar range, in spite of the structural simplification.[10h] This justifies the use of these synthetically less challenging analogues for SAR studies.

As part of our ongoing studies on nucleoside antibiotics, we currently examine variations in the muraymycin peptide unit.




Scheme 2. Previously reported syntheses of muraymycin analogues by Ichikawa et al. (**A**) and Ducho et al. (**B**) and novel approach of this work (**C**).

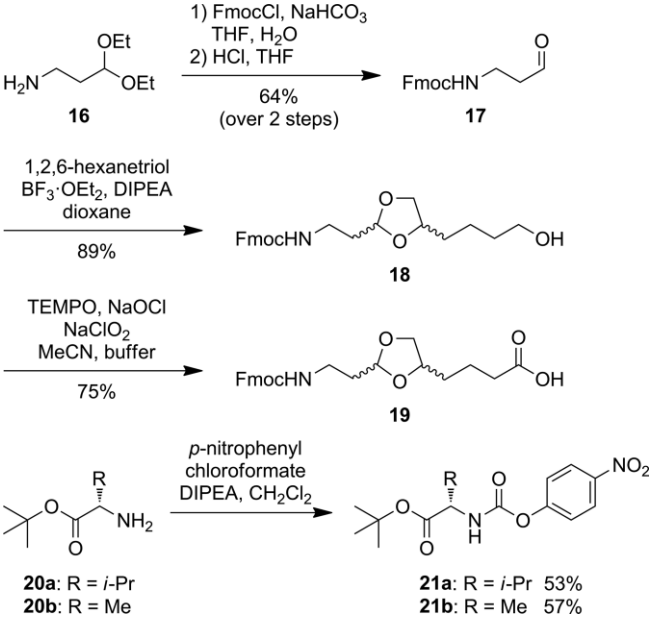
One goal is to elucidate which amino acids are essential for MraY inhibition and to what extent they contribute to the inhibitory activity. Thus, we now report an alanine scan of the peptide moiety, i.e. a one-by-one replacement of its three L-amino acids with L-alanine and subsequent biological evaluation of the resultant muraymycin analogues. To facilitate the synthesis, we have developed an alternative approach (Scheme 2, route C), i.e. a modular method based on solid phase-supported peptide synthesis (SPPS).

## Results and Discussion

Solid phase-supported approaches have been reported before for the mureidomycin/sansanmycin subclasses of uridine-derived nucleoside antibiotics, which are structurally related to muraymycins.[13] In their solid phase-supported synthesis of sansanmycins, Tran et al. have established the strategy to form the sansanmycin peptide unit on the solid phase and couple it to the nucleoside building block in solution at a later stage.[13b] For our novel approach towards muraymycins, we have similarly combined the solid phase-supported synthesis of the urea-containing peptide moiety **14** (via **15**) with late-stage connection to the nucleoside building block **13** (Scheme 2, route C). We have envisioned that this would enable rapid access to variations in the peptide unit. Furthermore, reactions involving the nucleoside building block **13** are reduced to a minimum because of its modular introduction in the penultimate step of the synthesis (prior to global deprotection). The 5′-defunctionalised variation **13** of the muraymycin nucleoside unit was chosen for the aforementioned reasons (vide supra).

It is known from literature precedent that a final global deprotection step under acidic conditions furnishes muraymycins and their analogues in a reliable and robust manner.[10,11] Hence, the overall strategy for the protection of amino acid side chains and the cleavage of the peptide unit from the solid support was conceived accordingly. For the envisioned solid phase-supported synthesis, we have chosen a trityl resin in combination with an Fmoc protecting group strategy. As Fmoc is a standard protecting group used in SPPS, well-established protocols are available.[14] The trityl resin is cleavable under mild acidic conditions and can thus be orthogonally used in combination with acid-sensitive side chain protecting groups such as Boc (which requires harsher conditions for cleavage). In contrast to standard SPPS, we intended to prepare a peptide-linker aldehyde **14** that would undergo reductive amination after its cleavage from the resin. Konno et al. reported the preparation of similar peptides on solid support,[15] but obtained epimers for the amino acid bearing the aldehyde functionality. However, this problem was impossible for our synthetic route because of the propyl linker between the first amino acid and the aldehyde functionality.

For appropriate functionalisation of the resin, we first converted 1-amino-3,3-diethoxypropane **16** into Fmoc-protected aldehyde **17** (Scheme 3). Thus, Fmoc protection and acidic cleavage of the acetal furnished **17** in 64 % yield over two steps. Aldehyde **17** was further transformed into 1,3-dioxolane **18** by acetalisation with 1,2,6-hexanetriol in 89 % yield. Subsequent metal-free oxidation of the primary alcohol **18** under TEMPO-Pinnick conditions afforded carboxylic acid **19** in 75 % yield. Acid **19** would later serve as a linker unit for the attachment to the trityl resin by tritylation of the carboxylate.

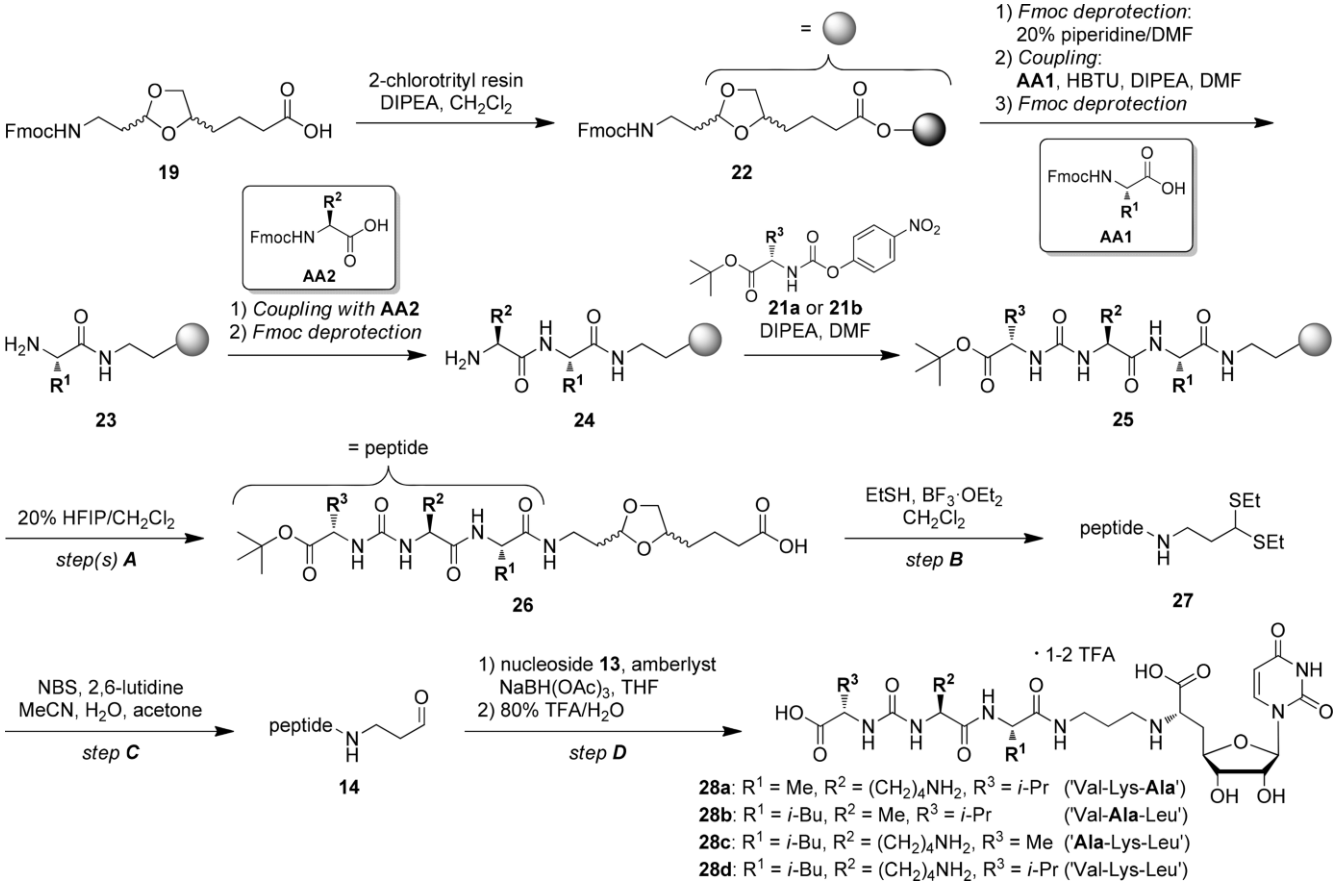



Scheme 3. Synthesis of the linker unit **19** and of building blocks **21a**,**b** for urea formation.

For the formation of the urea peptide motif on the solid support, *p*-nitrophenyl carbamates of the terminal amino acids were prepared as building blocks (Scheme 3). Thus, L-valine *tert*-butyl ester **20a** (R = *i*Pr) and L-alanine *tert*-butyl ester **20b** (R = Me), respectively, were treated with *p*-nitrophenyl chloroformate. This directly furnished the desired *p*-nitrophenyl carbamates **21a** (valine derivative, R = *i*Pr) and **21b** (alanine derivative, R = Me) in yields of 53 % and 57 %, respectively.

The solid phase-supported reaction sequence started with the attachment of the carboxylic acid **19** to the 2-chlorotrityl chloride resin, furnishing resin-bound, Fmoc-protected intermediate **22** (Scheme 4). Non-converted trityl groups on the resin were capped with a mixture of $CH_2Cl_2$, MeOH and DIPEA. For subsequent Fmoc deprotection, 20 % piperidine in DMF was added to the resin and the mixture was shaken thoroughly (2 × 10 min). Coupling of the first amino acid **AA1** was performed using an excess of 3.0 equivalents (eq) of the Fmoc- and side chain-protected amino acid, 3.0 eq of HBTU as coupling reagent and 6 eq of DIPEA in DMF, thus yielding resin-linked amino acid **23**. All coupling steps were performed twice. Reaction times between 2 and 40 h were tolerated, but at least one of the coupling steps was left for 12 h or longer to complete. Washing steps with DMF and $CH_2Cl_2$ were carried out after each deprotection and coupling step.

The next amino acid **AA2** was coupled using the same protocol (Scheme 4). After cleavage of the Fmoc group, resin-linked dipeptide **24** was furnished, which then underwent urea formation by treatment with *p*-nitrophenyl carbamate **21a** or **21b** in the presence of DIPEA in DMF. The resultant urea peptide **25** was then cleaved from the resin using 1,1,1,3,3,3-hexafluoro-2-




Scheme 4. SPPS of the muraymycin peptide unit and subsequent reactions towards muraymycin analogues **28a**–**d**. Steps: **A**: all resin-bound SPPS steps; **B**: transacetalisation; **C**: aldehyde formation; **D**: reductive amination and global deprotection. Yields for steps **A**–**D** are provided in Table 1.

propanol (HFIP), which left the acidically cleavable Boc protecting group and the *tert*-butyl ester intact. This afforded 1,3-dioxolane **26** as a stereoisomeric mixture at the dioxolane unit, which was treated with ethanethiol and boron trifluoride to give dithioacetal **27**. This transformation usually proceeded with good yields (67–85 %, vide infra), even though sometimes the formation of partially deprotected side products occurred and extended reaction periods of several days were necessary.

The thus obtained dithioacetal **27** was cleaved with *N*-bromosuccinimide and 2,6-lutidine[16] to regain the aldehyde functionality in peptides of type **14** (Scheme 4, also cf. Scheme 2). The resultant aldehyde was then used for reductive amination with the nucleoside building block **13**[12b] (cf. Scheme 2) using sodium borohydride and amberlyst as acidic activator. After global deprotection with aqueous trifluoroacetic acid and HPLC purification, the desired muraymycin analogues **28a**–**d** were obtained as TFA salts. The yields over the final two steps ranged from 39 to 58 % (vide infra). Considering that reductive aminations of similar systems have sometimes been problematic with respect to robustness and the occurrence of side products, these yields can be considered satisfying.

Target compound **28d** represents a previously reported simplified muraymycin analogue[10i] containing the 5′-defunctionalised version of the nucleoside core unit and the amino acid sequence valine-lysine-leucine (all-L) in the peptide moiety. Thus, it is an analogue of D-series muraymycins with lysine replacing the synthetically challenging epicapreomycidine unit. All other target compounds **28a**–**c** are novel and part of an alanine scan of the muraymycin peptide unit, with **28d** serving as the reference compound. Thus, in **28a** L-alanine replaces the native L-leucine, while in **28b** the L-lysine and in **28c** the L-valine motifs, respectively, are replaced by L-alanine.

Yields for the SPPS-based synthesis of **28a**–**d** are listed in Table 1 (also cf. Scheme 4). For step(s) **A** (combination of all resin-bound SPPS steps), they were in the range of 48–73 % and for step **B** (transacetalisation) in the range 67–85 % (vide supra). Step **C** (aldehyde formation) proceeded with 37–63 % yield and step **D** (reductive connection to the nucleoside and deprotection) with 39–52 % yield (including preparative HPLC purification of the target compounds, vide supra). Thus, overall yields were in the range of 9–14 %, demonstrating the effi-

Table 1. Yields [%] for synthetic steps **A**–**D** depicted in Scheme 4.

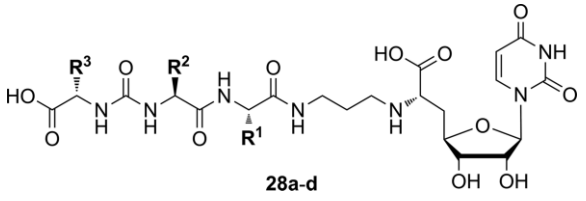
| Compound[a] | A | B | C | D | overall[b] |
|---|---|---|---|---|---|
| **28a** (Val-Lys-**Ala**) | 67 | 72 | 55 | 39 | **10** |
| **28b** (Val-**Ala**-Leu) | 53 | 85 | 54 | 58 | **14** |
| **28c** (**Ala**-Lys-Leu) | 48 | 74 | 63 | 46 | **10** |
| **28d** (Val-Lys-Leu) | 73 | 67 | 37 | 52 | **9** |

[a] In parentheses: amino acid sequence in order $R^3$-$R^2$-$R^1$ (see Scheme 4), with positions of alanine replacements (relative to **28d**) highlighted in bold. [b] Overall yields from precursor **19**.

ciency of the novel SPPS-based route with respect to the significant number of transformations and the facileness of purification.

The novel target compounds **28a**–**c** were investigated for their inhibitory potency towards the bacterial target protein MraY, using an established fluorescence-based in vitro assay for MraY activity.[17] For this assay, a crude membrane preparation of MraY from *S. aureus* (heterologously overexpressed in *E. coli*) was employed as described before.[10f,10h,10i,10j,17] The results thus obtained, including the previously reported inhibitory potency of reference compound **28d**,[10i] are given in Table 2.

Table 2. In vitro inhibitory potencies ($IC_{50}$ values in μM)[a] of muraymycin analogues **28a**–**d** towards the bacterial target protein MraY.



**28a-d**

| **28a** (Val-Lys-**Ala**)[b] | **28b** (Val-**Ala**-Leu)[b] | **28c** (**Ala**-Lys-Leu)[b] | **28d** (Val-Lys-Leu)[b] |
|---|---|---|---|
| 95 ± 40 | 14 ± 2 | 21 ± 4 | 2.5 ± 0.6 |

[a] Mean ±SD of triplicate measurements. [b] In parentheses: amino acid sequence in order $R^3$-$R^2$-$R^1$, with positions of alanine replacements (relative to **28d**) highlighted in bold.

All novel analogues **28a**–**c** showed some inhibitory activity towards MraY in the μM range, even though their potencies were lower than the activity of reference compound **28d**. The formal replacement of the central L-leucine with L-alanine (**28a**) gave the most pronounced loss of inhibitory potency (ca. 40-fold) relative to **28d**. In contrast, exchanging the cationic L-lysine motif (**28b**) and the terminal L-valine unit (**28c**) with L-alanine only reduced the inhibitory activities ca. 6-fold (for **28b**) and ca. 8-fold (for **28c**), respectively. Hence, the alanine scan approach revealed that the central L-leucine residue plays the most essential role of all three amino acid moieties in the binding of muraymycin analogues to MraY, probably due to hydrophobic interactions with the protein in this position.

It should be noted that these results were obtained using a simplified (5′-deoxy) version of the muraymycin scaffold. However, the fact that such 5′-deoxy analogues can display low-μM to nM[10h] activities as MraY inhibitors demonstrate their validity as surrogates for naturally occurring muraymycins. This strongly suggests that the obtained insights into the interaction of **28a**–**c** with MraY will also be valid for the parent natural products. Furthermore, the results are in good agreement with the current knowledge on MraY and its inhibition. It is known that the protein shows pronounced conformational plasticity and that the muraymycin peptide unit is accommodated in a rather solvent-exposed position on the surface of the active site, rather than in a defined pocket.[9] This is reflected in the observation that none of the three amino acid replacements with L-alanine led to a dramatic loss of inhibitory potency towards MraY as no key interaction seems to be mediated by any of the three side chains. It can also be assumed that the protein can most likely conformationally adapt to moderate changes in the structure of the peptide unit.

We had reported before that the simplified muraymycin analogue **28d** is not antibacterially active in cellulo (MIC > 50 μg/mL against *E. coli*), probably as a result of its moderately potent target interaction in combination with limited cellular uptake.[10i] Therefore, we have not studied novel analogues **28a**–**c** for potential antibacterial effects in cellulo as they were weaker MraY inhibitors than **28d**. Thus, **28a**–**c** served as tool compounds to map MraY-ligand interactions for muraymycin-derived MraY inhibitors rather than as candidate compounds for muraymycin analogues with improved antibacterial activities.

## Conclusions

In summary, we have successfully established a novel solid phase-supported strategy for the synthesis of muraymycin analogues. Our novel approach is modular and efficient, as demonstrated by the facile preparation of three novel and one previously reported muraymycin analogue(s) for an alanine scan of the muraymycin peptide unit. In vitro studies on the inhibitory activities of these compounds **28a**–**d** towards the bacterial target protein MraY revealed that all alanine-containing analogues were still reasonably potent MraY inhibitors. However, MraY inhibition was most hampered when the central L-leucine motif of the muraymycin scaffold was formally exchanged to L-alanine. This indicated that the leucine unit rather strongly contributes to the interaction with MraY, most likely due to hydrophobic contacts.

Our results are useful to guide the future design of muraymycin analogues. Firstly, they suggest that the central L-leucine or (*O*-acylated) 3-hydroxy-L-leucine motif, respectively, should either be retained or only be incrementally altered. Secondly, it is obvious that the valine and lysine units may be exchanged into different amino acids. This might be useful in the design of muraymycin conjugates or tagged muraymycin analogues as probes to further study the cellular interactions of this class of compounds. The SPPS strategy should also enable the introduction of amino acids with accordingly functionalised side chains, even though this might require the adjustment of some reaction conditions.

Overall, the reported efficient SPPS-based methodology for the synthesis of muraymycin analogues will thereby enable further variations in the muraymycin peptide unit. With respect to the modular nature of this novel synthetic strategy, variations in the nucleoside moiety will also be feasible. This will facilitate more detailed SAR studies on this promising class of antibacterial agents, and work along this line is currently on the way in our laboratories.

## Experimental Section

**General Methods:** Chemicals were purchased from standard suppliers and used without further purification. Reactions involving oxygen- and/or moisture-sensitive reagents were carried out under an atmosphere of nitrogen using anhydrous solvents. The glass

equipment used for these reactions was dried by heating prior to use. Anhydrous solvents were obtained in the following manner: $CH_2Cl_2$, THF and DMF were purchased in HPLC quality and dried with a solvent purification system (MBRAUN MB SPS 800). MeOH was of absolute quality, degassed and stored over activated molecular sieves (3 Å). $NEt_3$ was of absolute quality, degassed and stored over activated molecular sieves (4 Å). Solvents for reactions without inert conditions, extractions, and chromatography were of technical quality and distilled prior to their use. All other solvents were of p.a. quality, and distilled water was used throughout. Column chromatography was carried out on silica gel 60 (0.040–0.063 mm, 230–400 mesh ASTM, VWR) under flash conditions. TLC was performed on aluminium plates precoated with silica gel 60 $F_{254}$ (VWR). Visualisation of the spots was carried out using UV light (254 nm) where appropriate and/or staining under heating [$KMnO_4$ staining solution: 1 g of $KMnO_4$, 6 g of $K_2CO_3$ and 1.5 mL of 5 % $NaOH_{aq}$ (w/v), all dissolved in 100 mL of $H_2O$; ninhydrin staining solution: 0.3 g of ninhydrin, 3 mL of AcOH, all dissolved in 100 mL of 1-butanol, $H_2SO_4$ staining solution: 4 g of vanillin, 25 mL of conc. $H_2SO_4$, 80 mL of AcOH, all dissolved in 680 mL of MeOH]. Semipreparative HPLC was performed on an Agilent Technologies 1200 Series system equipped with an MWD detector (254.16/280.16) and a LiChroCart™ column (10 × 250 mm) containing reversed phase silica gel Purospher™ RP18e (5 µm, VWR). Method 1: eluent A water (+0.1 % TFA), eluent B MeCN (+0.1 % TFA); 0–25 min gradient of B (3–30 %), 25–30 min gradient of B (30–100 %), 30–35 min 100 % B, 35–36 min gradient of B (100–3 %); flow 3 mL/min. Method 2: eluent A water (+0.1 % TFA), eluent B MeCN (+0.1 % TFA); 0–25 min gradient of B (2–20 %), 25–30 min gradient of B (20–100 %), 30–35 min 100 % B, 35–36 min gradient of B (100–2 %); flow 3 mL/min. 500 MHz-$^1$H NMR, 126 MHz-$^{13}$C NMR and 376 MHz-$^{19}$F NMR spectra were recorded on Bruker AVANCE-500 spectrometers. All $^{13}$C NMR spectra are $^1$H-decoupled. All spectra were recorded at room temperature and were referenced internally to solvent reference frequencies. Chemical shifts ($\delta$) are quoted in ppm. Coupling constants ($J$) are reported in Hz to the nearest 0.1 Hz. The assignment of signals was carried out using $^1$H,$^1$H-COSY, HSQC and HMBC spectra obtained on the spectrometers mentioned above. High resolution (HR) ESI mass spectrometry was carried out on a Dionex UltiMate 3000 HPLC system and on a Bruker time-of-flight (TOF) maXis. Infrared spectroscopy (IR) was performed on a Bruker Vertex 70 spectrometer equipped with an integrated ATR unit (PlatinumATR™). Wavenumbers ($\tilde{\nu}$) are quoted in cm$^{-1}$. UV spectroscopy was carried out on an Agilent Cary 100 spectrophotometer. Wavelengths of maximum absorption ($\lambda_{max}$) are reported in nm.

**General Procedure (GP1) for the synthesis of *p*-nitrophenyl carbamates:** The respective amino acid *tert*-butyl ester **20** (1.0 equiv.) was dissolved in $CH_2Cl_2$, and DIPEA (1.0 equiv.) and *p*-nitrophenyl chloroformate (1.2 equiv.) were added. The mixture was stirred at r.t. overnight. The solvent was evaporated under reduced pressure and the resultant crude product was purified by column chromatography to give the respective *p*-nitrophenyl carbamate **21**.

**General Procedure (GP2) for solid phase-supported peptide synthesis:** 2-Chlorotrityl chloride resin (1.0–1.3 equiv.) was weighed into a syringe equipped with a filter frit, pre-dried and allowed to swell in $CH_2Cl_2$ for 30–60 min. *N*-Fmoc-protected carboxylic acid **19** (1.0 equiv.) and DIPEA (3.0 equiv.) were dissolved in $CH_2Cl_2$ and added to the resin, and the mixture was shaken at r.t. for several hours. The solution was then filtered off, the resin was washed with DMF, $CH_2Cl_2$ and DMF again (5 × 2–4 mL each). A solution of $CH_2Cl_2$, MeOH and DIPEA (17:2:1) was added to the resin, and the mixture was shaken for 5 min to cap remaining trityl chloride moieties. The resin was then washed again with DMF, $CH_2Cl_2$ and DMF. Fmoc deprotection was carried out by shaking the resin with a solution of 20 % piperidine in DMF for 10 min. It was then washed with DMF, $CH_2Cl_2$ and DMF (5 × 2–4 mL each). The procedure was carried out twice. The *N*-Fmoc-protected amino acid **AA1** (6 equiv.), HBTU (6 equiv.) and DIPEA (12 equiv.) were dissolved in DMF under inert gas conditions. The solution was added to the resin in two portions, and the mixture was shaken at r.t. for 1–4 h for the first coupling and 16–40 h for the second. The resin was then washed with DMF, $CH_2Cl_2$ and DMF (5 × 2–4 mL each). Fmoc deprotection and coupling with the second amino acid **AA2** were carried out in the same manner. For urea formation, the *p*-nitrophenyl carbamate of the respective amino acid (2.0 equiv.) and DIPEA (4.0 equiv.) were dissolved in DMF. The solution was added to the resin, and the mixture was shaken at r.t. for 6 h and then washed with DMF and $CH_2Cl_2$ (5 × 2–4 mL each).

**General Procedure (GP3) for cleavage from the resin:** A solution of 20 % HFIP in $CH_2Cl_2$ was added to the resin, and the mixture was shaken at r.t. for 1 h. The resin was then washed 10–15 times with $CH_2Cl_2$ (2–4 mL each). The combined solutions were evaporated under reduced pressure to give the respective carboxylic acid **26**.

**General Procedure (GP4) for dithioacetal formation:** The respective 1,3-dioxolane **26** (1.0 equiv.) was dissolved in $CH_2Cl_2$ and boron trifluoride diethyl etherate (0.10–0.15 equiv.) and ethanethiol (15–20 equiv.) were added. The mixture was stirred at r.t. for several days and boron trifluoride diethyl etherate was added if necessary. When the reaction was complete, DIPEA (6.0 equiv.) was added, and the reaction mixture was washed with water (2 ×) and brine (2 ×). The organic layer was dried with $Na_2SO_4$, the solvent was evaporated under reduced pressure and the resultant crude product was purified by column chromatography to give the respective dithioacetal **27**.

**General Procedure (GP5) for thioacetal cleavage:** The respective dithioacetal **27** (1.0 equiv.) was dissolved in a mixture of MeCN, water and acetone (8:2:1). At 0 °C, NBS (8 equiv.) and 2,6-lutidine (16 equiv.) were added, the reaction mixture was stirred for 5–10 min, and the reaction was then quenched with sat. $Na_2S_2O_3$ solution (10–20 mL). The aqueous layer was extracted with $CH_2Cl_2$ (3 ×), and the combined organics were washed with brine (1 ×) and dried with $Na_2SO_4$. The solvent was evaporated under reduced pressure and the resultant crude product was purified by column chromatography.

**General Procedure (GP6) for reductive amination:** The respective peptide aldehyde **14** (1.0 equiv.) was dissolved in THF over molecular sieves (4 Å), nucleoside **13**[12b] (1.2 equiv.) was added and the mixture was stirred at r.t. for 1 d. Then, amberlyst-15™ (spatula tip) and sodium triacetoxyborohydride (2.0 equiv.) were added and the mixture was stirred at r.t. overnight. The reaction mixture was filtered and the insoluble material was washed with EtOAc. The combined filtrates were washed with sat. $Na_2CO_3$ solution (1 ×), and the aqueous layer was extracted with EtOAc (1 ×). The combined organics were dried with $Na_2SO_4$, the solvent was evaporated under reduced pressure and the resultant crude product was purified by column chromatography to give the respective fully protected muraymycin analogue.

**Val-Lys-Ala peptide aldehyde (14a):** General procedure **GP5** with Val-Lys-Ala peptide dithioacetal **27a** (8.3 mg, 12 µmol), NBS (19 mg, 0.11 mmol) and 2,6-lutidine (23 µL, 21 mg, 0.20 mmol) in MeCN, water and acetone (2.2 mL) over 5 min. Column chromatography ($CH_2Cl_2$/MeOH, 99:1 → 98:2 → 95:5) gave **14a** as a colourless solid (3.8 mg, 55 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.86 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 0.92 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 1.27–1.50 (m, 4H, Lys-

4-H, Lys-5-H), 1.34 (d, $J$ = 7.2 Hz, 3H, Ala-3-H), 1.45 (s, 9H, Boc-OC($CH_3$)$_3$), 1.47 (s, 9H, OC($CH_3$)$_3$), 1.75–1.81 (m, 2H, Lys-3-H), 2.08–2.14 (m, 1H, Val-2-H), 2.70 (dt, $J$ = 6.3, 1.1 Hz, 2H, 2-H), 3.06–3.13 (m, 2H, Lys-6-H), 3.48–3.60 (m, 2H, 3-H), 4.18–4.21 (m, 1H, Lys-2-H), 4.29–4.31 (m, 1H, Val-2-H), 4.43–4.49 (m, 1H, Ala-2-H), 4.99 (s, 1H, Boc-NH), 5.72 (d, $J$ = 8.2 Hz, 1H, Val-NH), 6.20 (s, 1H, Lys-NH), 6.87 (d, $J$ = 6.1 Hz, 1H, Ala-NH), 7.38 (s, 1H, 3-NH), 9.77 (t, $J$ = 1.1 Hz, 1H, 1-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 17.67 (Ala-C-3), 18.10 (Val-C-4), 19.20 (Lys-C-4), 28.22 [OC($CH_3$)$_3$], 28.65 (Boc-OC($CH_3$)$_3$), 29.84 (Lys-C-5), 31.69 (Lys-C-3), 33.34 (C-3), 39.62 (Lys-C-6), 43.69 (C-2), 49.19 (Ala-C-2), 54.74 (Lys-C-2), 58.44 (Val-C-2), 79.10 (Boc-OC($CH_3$)$_3$), 82.02 [OC($CH_3$)$_3$], 156.88 (Boc-C=O), 158.50 (urea-C=O), 172.31–173.12 (Ala-C-1, Lys-C-1, Val-C-1), 201.36 (C-1) ppm. HRMS (ESI): calcd. for $C_{27}H_{50}N_5O_8$ [M + H]$^+$ 572.3654, found 572.3657. IR (ATR): $\tilde{\nu}$ = 2970, 1737, 1626, 1541, 1366, 1229, 1217, 1157 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 222 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.33.

**Val-Ala-Leu peptide aldehyde (14b):** General procedure **GP5** with Val-Ala-Leu peptide dithioacetal **27b** (20.6 mg, 36.6 μmol), NBS (52.5 mg, 0.295 mmol) and 2,6-lutidine (70 μL, 64 mg, 0.60 mmol) in MeCN, water and acetone (4.4 mL) over 7 min. Column chromatography ($CH_2Cl_2$/MeOH, 99:1 → 98:2 → 95:5) gave **14b** as a colourless solid (9.0 mg, 54 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.86–0.92 (m, 12H, Val-4-H, Leu-5-H), 1.29 (d, $J$ = 7.0 Hz, 3H, Ala-3-H), 1.46 (s, 9H, OC($CH_3$)$_3$), 1.51–1.69 (m, 3H, Leu-3-H, Leu-4-H), 2.03–2.09 (m, 1H, Val-3-H), 2.70–2.72 (m, 2H, 2-H), 3.48–3.55 (m, 1H, 3-H$_a$), 3.57–3.63 (m, 1H, 3-H$_b$), 4.28 (dd, $J$ = 8.8, 4.9 Hz, 1H, Val-2-H), 4.51–4.56 (m, 1H, Leu-2-H), 4.56–4.61 (m, 1H, Ala-2-H), 6.13 (d, $J$ = 8.8 Hz, 1H, Val-NH), 6.44 (s, 1H, Ala-NH), 7.53 (s, 1H, Leu-NH), 7.89 (s, 1H, 3-NH), 9.76 (s, 1H, 1-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 17.91 (Val-C-4), 19.13 (Val-C-4), 19.73 (Ala-C-3), 22.43 (Leu-C-5), 22.80 (Leu-C-5), 24.93 (Leu-C-4), 28.24 [OC($CH_3$)$_3$], 31.94 (Val-C-3), 33.38 (C-3), 41.34 (Leu-C-3), 43.77 (C-2), 49.51 (Ala-C-2), 52.03 (Leu-C-2), 58.47 (Val-C-2), 81.81 [OC($CH_3$)$_3$], 157.88 (urea-C=O), 172.69 (Val-C-1), 172.85 (Leu-C-1), 174.11 (Ala-C-1), 201.15 (C-1) ppm. HRMS (ESI): calcd. for $C_{22}H_{41}N_4O_6$ [M + H]$^+$ 457.3021, found 457.3021. IR (ATR): $\tilde{\nu}$ = 3279, 2969, 1734, 1629, 1542, 1368, 1217, 1148 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 223 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.27.

**Ala-Lys-Leu peptide aldehyde (14c):** General procedure **GP5** with Ala-Lys-Leu peptide dithioacetal **27c** (18.4 mg, 26.6 μmol), NBS (38.8 mg, 0.218 mmol) and 2,6-lutidine (50 μL, 46 mg, 0.43 mmol) in MeCN, water and acetone (3.3 mL) over 5 min. Column chromatography ($CH_2Cl_2$/MeOH, 99:1 → 98:2 → 95:5) gave **14c** as a colourless solid (9.8 mg, 63 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.88 (d, $J$ = 6.6 Hz, 3H, Leu-5-H), 0.91 (d, $J$ = 6.6 Hz, 3H, Leu-5-H), 1.32–1.37 (m, 2H, Lys-4-H), 1.33 (d, $J$ = 7.2 Hz, 3H, Ala-3-H), 1.44 (s, 9H, Boc-OC($CH_3$)$_3$), 1.46 (s, 9H, OC($CH_3$)$_3$), 1.46–1.77 (m, 7H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H), 2.69 (t, $J$ = 6.4 Hz, 2H, 2-H), 3.03–3.13 (m, 2H, Lys-6-H), 3.51–3.57 (m, 2H, 3-H), 4.28–4.33 (m, 1H, Lys-2-H), 4.31–4.37 (m, 1H, Ala-2-H), 4.47–4.52 (m, 1H, Leu-2-H), 5.02 (s, 1H, Boc-NH), 5.92 (s, 1H, Ala-NH), 6.41 (s, 1H, Lys-NH), 7.02 (s, 1H, Leu-NH), 7.65 (s, 1H, 3-NH), 9.76 (s, 1H, 1-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 19.33 (Ala-C-3), 22.03 (Leu-C-5), 22.29 (Lys-C-4), 23.03 (Leu-C-5), 25.03 (Leu-C-4), 28.14 [OC($CH_3$)$_3$], 28.63 (Boc-OC($CH_3$)$_3$), 29.78 (Lys-C-5), 32.12 (Lys-C-3), 33.40 (C-3), 39.67 (Lys-C-6), 40.81 (Leu-C-3), 43.66 (C-2), 49.53 (Ala-C-2), 51.94 (Leu-C-2), 54.38 (Lys-C-2), 79.39 (Boc-OC($CH_3$)$_3$), 81.83 [OC($CH_3$)$_3$], 156.80 (Boc-C=O), 157.99 (urea-C=O), 172.67 (Leu-C-1), 173.27 (Lys-C-1), 173.48 (Ala-C-1), 201.35 (C-1) ppm. HRMS (ESI): calcd. for $C_{28}H_{52}N_5O_8$ [M + H]$^+$ 586.3810, found 586.3816. IR (ATR): $\tilde{\nu}$ = 3275, 2970, 2934, 1737, 1628, 1541, 1366, 1229, 1217, 1158 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 222 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.21.

**Val-Lys-Leu peptide aldehyde (14d):** General procedure **GP5** with Val-Lys-Leu peptide dithioacetal **27d** (26.9 mg, 37.4 μmol), NBS (53.2 mg, 0.299 mmol) and 2,6-lutidine (70 μL, 64 mg, 0.60 mmol) in MeCN, water and acetone (4.4 mL) over 6 min. Column chromatography ($CH_2Cl_2$/MeOH, 99:1 → 98:2 → 95:5) gave **14d** as a colourless solid (8.5 mg, 37 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.85–0.91 (m, 12H, Val-4-H, Leu-5-H), 1.31–1.37 (m, 2H, Lys-4-H), 1.44 (s, 9H, Boc-OC($CH_3$)$_3$), 1.47 (s, 9H, OC($CH_3$)$_3$), 1.46–1.77 (m, 7H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H), 2.06–2.12 (m, 1H, Val-3-H), 2.68 (t, $J$ = 6.4 Hz, 2H, 2-H), 3.01–3.15 (m, 2H, Lys-6-H), 3.47–3.59 (m, 2H, 3-H), 4.27–4.31 (m, 2H, Val-2-H, Lys-2-H), 4.39–4.44 (m, 1H, Leu-2-H), 5.05 (s, 1H, Boc-NH), 5.86 (d, $J$ = 6.8 Hz, 1H, Val-NH), 6.32 (s, 1H, Lys-NH), 6.86 (s, 1H, Leu-NH), 7.57 (s, 1H, 3-NH), 9.75 (s, 1H, 1-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 17.76 (Val-C-4), 19.16 (Val-C-4), 22.04 (Leu-C-5), 22.24 (Lys-C-4), 22.99 (Leu-C-5), 25.01 (Leu-C-4), 28.23 [OC($CH_3$)$_3$], 28.63 (Boc-OC($CH_3$)$_3$), 29.79 (Lys-C-5), 31.81 (Val-C-3), 31.96 (Lys-C-3), 33.39 (C-3), 39.67 (Lys-C-6), 40.77 (Leu-C-3), 43.71 (C-2), 52.19 (Leu-C-2), 54.36 (Lys-C-2), 58.41 (Val-C-2), 79.34 (Boc-OC($CH_3$)$_3$), 81.99 [OC($CH_3$)$_3$], 156.77 (Boc-C=O), 158.45 (urea-C=O), 172.53 (Lys-C-1), 172.59 (Leu-C-1), 173.42 (Val-C-1), 201.27 (C-1) ppm. HRMS (ESI): calcd. for $C_{30}H_{56}N_5O_8$ [M + H]$^+$ 614.4123, found 614.4127. IR (ATR): $\tilde{\nu}$ = 1970, 1737, 1628, 1542, 1437, 1229, 1217, 1093 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 223 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.34.

***N*-Fmoc-3-aminopropanal (17):** A solution of 1-amino-3,3-diethoxypropane **16** (1.0 mL, 0.91 g, 6.2 mmol) and $NEt_3$ (1.7 mL, 1.3 g, 12 mmol) in $CH_2Cl_2$ (13 mL) was cooled to 0 °C. Fmoc-chloride (3.19 g, 12.4 mmol) was added and the mixture was stirred at r.t. for 7 d. The solution was then washed with $NH_4Cl$ solution (2 × 50 mL), $NaHCO_3$ solution (50 mL) and brine (50 mL), dried with $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The resultant crude product was purified by column chromatography (petroleum ether/EtOAc, 8:2 → 7:3) to give a mixture of *N*-Fmoc-1-amino-3,3-diethoxypropane and aldehyde **17** (2.18 g, 100 % yield: 2.28 g). This mixture was dissolved in THF (30 mL) and HCl (1 M, 6.0 mL, 5.9 mmol) was added. The mixture was stirred at r.t. for 5 h. Sat. $NaHCO_3$ solution (200 mL) was then added, the aqueous layer was extracted with EtOAc (3 × 150 mL) and the organic layer was dried with $Na_2SO_4$. The solvent was evaporated under reduced pressure and the resultant crude product was purified by column chromatography (petroleum ether/EtOAc, 9:1 → 5:1 → 4:1 → 1:1) to give **17** as a colourless solid (1.17 g, 64 % over 2 steps). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 2.74 (t, $J$ = 5.8 Hz, 2H, 2-H), 3.49 (dt, $J$ = 5.8, 5.8 Hz, 2H, 3-H), 4.20 (t, $J$ = 7.0 Hz, 1H, Fmoc-9-H), 4.39 (d, $J$ = 7.0 Hz, 2H, Fmoc-$CH_2$), 5.18 (s, 1H, 3-NH), 7.31 (t, $J$ = 7.5 Hz, 2H, Fmoc-3-H, Fmoc-6-H), 7.40 (t, $J$ = 7.5 Hz, 2H, Fmoc-2-H, Fmoc-7-H), 7.57 (d, $J$ = 7.5 Hz, 2H, Fmoc-4-H, Fmoc-5-H), 7.76 (d, $J$ = 7.5 Hz, 2H, Fmoc-1-H, Fmoc-8-H), 9.81 (s, 1H, 1-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 34.56 (C-3), 44.17 (C-2), 47.34 (Fmoc-C-9), 66.82 (Fmoc-$CH_2$), 120.12 (Fmoc-C-1, Fmoc-C-8), 125.15 (Fmoc-C-4, Fmoc-C-5), 127.16 (Fmoc-C-3, Fmoc-C-6), 127.83 (Fmoc-C-2, Fmoc-C-7), 141.44 (Fmoc-C-1a, Fmoc-C-8a), 143.99 (Fmoc-C-4a, Fmoc-C-5a), 156.45 (Fmoc-C=O), 201.40 (C-1) ppm. HRMS (ESI): calcd. for $C_{18}H_{18}NO_3$ [M + H]$^+$ 296.1281, found 296.1277. IR (ATR): $\tilde{\nu}$ = 3321, 2946, 1687, 1536, 1446, 1258, 1145, 732 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 267, 290, 301 nm. TLC (petroleum ether/EtOAc, 1:1): $R_f$ = 0.25.

**1,3-Dioxolane (18):** A solution of 1,2,6-hexanetriol (316 mg, 2.36 mmol), *N*-Fmoc-3-aminopropanal **17** (327 mg, 1.11 mmol) and boron trifluoride diethyl etherate (14 μL, 16 mg, 0.11 mmol) in 1,4-dioxane (18 mL) was stirred at r.t. for 20 h. DIPEA (0.29 mL, 0.22 g, 1.7 mmol) and EtOAc (100 mL) were then added. The organic layer was washed with water (2 × 100 mL) and brine (2 × 100 mL), dried with $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The resultant crude product was purified by column chroma-

tography (petroleum ether/EtOAc, 1:1) to give **18** as a colourless solid as a mixture of two diastereomers (*d.r.* 7:3, 409 mg, 89 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.39–1.71 (m, 2 × 6H, 3′-H, 4′-H, 5′-H), 1.88–1.94 (m, 2 × 2H, 2-H), 3.36–3.40 (m, 2 × 2H, 3-H), 3.46 (t, $J$ = 7.5 Hz, 1H, 1′-$H_a$), 3.53 (t, $J$ = 7.1 Hz, 1H, 1′-$H_a$), 3.63–3.67 (m, 2 × 2H, 6′-H), 3.95 (t, $J$ = 7.1 Hz, 1H, 1′-$H_b$), 4.03–4.11 (m, 2 × 1H, 2′-H), 4.13–4.17 (m, 1H, 1′-$H_b$), 4.21–4.24 (m, 2 × 1H, Fmoc-9-H), 4.37 (d, $J$ = 7.1 Hz, 2 × 2H, Fmoc-$CH_2$), 4.98–5.00 (m, 1H, 1-H), 5.05–5.07 (m, 1H, 1-H), 5.29–5.32 (m, 2 × 1H, 3-NH), 7.31 (t, $J$ = 7.5 Hz, 2 × 2H, Fmoc-3-H, Fmoc-6-H), 7.40 (t, $J$ = 7.5 Hz, 2 × 2H, Fmoc-2-H, Fmoc-7-H), 7.60 (d, $J$ = 7.5 Hz, 2 × 2H, Fmoc-4-H, Fmoc-5-H), 7.76 (d, $J$ = 7.5 Hz, 2 × 2H, Fmoc-1-H, Fmoc-8-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 22.17, 22.23 (C-4′), 32.59–33.59 (C-2, C-3′, C-5′), 36.39 (C-3), 47.43 (Fmoc-C-9), 62.70, 62.75 (C-6′), 66.76 (Fmoc-$CH_2$), 69.82, 70.69 (C-1′), 76.31 (C-2′), 102.90, 103.63 (C-1), 120.10 (Fmoc-C-1, Fmoc-C-8), 125.23 (Fmoc-C-4, Fmoc-C-5), 127.14 (Fmoc-C-3, Fmoc-C-6), 127.78 (Fmoc-C-2, Fmoc-C-7), 141.44 (Fmoc-C-1a, Fmoc-C-8a), 144.18 (Fmoc-C-4a, Fmoc-C-5a), 156.47 (Fmoc-C=O) ppm. HRMS (ESI): calcd. for $C_{24}H_{30}NO_5$ [M + H]$^+$ 412.2118, found 412.2118. IR (ATR): $\tilde{\nu}$ = 3331, 2934, 2861, 1683, 1534, 1261, 1004, 620 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 267, 290, 301 nm. TLC (petroleum ether/EtOAc, 3:7): $R_f$ = 0.15.

**1,3-Dioxolane carboxylic acid (19):** 1,3-Dioxolane **18** (358 mg, 0.870 mmol) was dissolved in MeCN and phosphate buffer (5:3, phosphate buffer 0.2 M, pH≈ 7, 16 mL). TEMPO (42.1 mg, 0.269 mmol), $NaClO_2$ (156 mg, 1.72 mmol) and NaOCl solution (5 % in water, 0.12 mL) were added and the reaction mixture was stirred at 35 °C for 3 d. The mixture was then cooled to r.t. and EtOAc (120 mL) was added. The organic layer was washed with sat. $Na_2S_2O_3$ solution (2 × 120 mL) and brine (2 × 120 mL), dried with $Na_2SO_4$ and the solvent was evaporated under reduced pressure. The resultant crude product was purified by column chromatography ($CH_2Cl_2$/MeOH, 95:5) to give **19** as a colourless, wax-like oil (279 mg, 75 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.62–1.83 (m, 2 × 4H, 3′-H, 4′-H), 1.85–1.92 (m, 2 × 2H, 2-H), 2.39–2.43 (m, 2 × 2H, 5′-H), 3.32–3.39 (m, 2 × 2H, 3-H), 3.43–3.55 (m, 2 × 1H, 1′-$H_a$), 3.94 (t, $J$ = 7.2 Hz, 1H, 1′-$H_b$), 4.04–4.12 (m, 2 × 2H, 2′-H), 4.13–4.16 (m, 1H, 1′-$H_b$), 4.21–4.24 (m, 2 × 1H, Fmoc-9-H), 4.37–4.42 (m, 2 × 2H, Fmoc-$CH_2$), 4.94–4.98 (m, 1H, 1-H), 5.04–5.05 (m, 1H, 1-H), 5.29–5.30 (m, 2 × 1H, 3-NH), 7.29–7.32 (m, 2 × 2H, Fmoc-3-H, Fmoc-6-H), 7.39 (t, $J$ = 7.5 Hz, 2 × 2H, Fmoc-2-H, Fmoc-7-H), 7.59–7.60 (m, 2 × 2H, Fmoc-4-H, Fmoc-5-H), 7.76 (d, $J$ = 7.5 Hz, 2 × 2H, Fmoc-1-H, Fmoc-8-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 21.10, 21.19 (C-4′), 32.84–33.61 (C-2, C-3′, C-5′), 36.37 (C-3), 47.39 (Fmoc-C-9), 66.80 (Fmoc-$CH_2$), 69.73 (C-1′), 75.95, 76.44 (C-2′), 102.91, 103.68 (C-1), 120.08 (Fmoc-C-1, Fmoc-C-8), 125.21 (Fmoc-C-4, Fmoc-C-5), 127.13 (Fmoc-C-3, Fmoc-C-6), 127.77 (Fmoc-C-2, Fmoc-C-7), 141.42 (Fmoc-C-1a, Fmoc-C-8a), 144.16 (Fmoc-C-4a, Fmoc-C-5a), 156.54 (Fmoc-C=O), 178.22 (C-6′) ppm. HRMS (ESI): calcd. for $C_{24}H_{28}NO_6$ [M + H]$^+$ 426.1911, found 426.1909. IR (ATR): $\tilde{\nu}$ = 3338, 2942, 2874, 1684, 1532, 1258, 1138, 1005, 738 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 267, 290, 301 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.47.

***tert*-Butyl [(4-nitrophenoxy)carbonyl]-L-valinate (21a):** General procedure **GP1** with L-valine *tert*-butyl ester hydrochloride (489 mg, 2.33 mmol), DIPEA (0.40 mL, 0.30 g, 2.3 mmol) and *p*-nitrophenyl chloroformate (568 mg, 2.82 mmol) in $CH_2Cl_2$ (10 mL). The reaction mixture was stirred for 2 d. Column chromatography (petroleum ether/EtOAc, 7:1) gave **21a** as a colourless oil (416 mg, 53 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.96 (d, $J$ = 6.9 Hz, 3H, 4-H), 1.03 (d, $J$ = 6.9 Hz, 3H, 4-H), 1.50 (s, 9H, OC($CH_3$)$_3$), 2.21–2.28 (m, 1H, 3-H), 4.24 (dd, $J$ = 9.0, 4.3 Hz, 1H, 2-H), 5.67 (d, $J$ = 9.0 Hz, 1H, NH), 7.33 (d, $J$ = 9.2 Hz, 2H, 2′-H, 6′-H), 8.24 (d, $J$ = 9.2 Hz, 2H, 3′-H, 5′-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 17.46 (C-4), 19.07 (C-4), 28.19 [OC($CH_3$)$_3$], 31.62 (C-3), 59.63 (C-2), 82.75 [O*C*($CH_3$)$_3$], 122.09 (C-2′, C-6′), 125.25 (C-3′, C-5′), 144.94 (C-4′), 153.18 (carbamate-C=O), 155.98 (C-1′), 170.75 (C-1) ppm. HRMS (ESI): calcd. for $C_{16}H_{23}N_2O_6$ [M + H]$^+$ 339.1551, found 339.1549. IR (ATR): $\tilde{\nu}$ = 3339, 2969, 1719, 1521, 1486, 1343, 1204, 1144, 859 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 277 nm. TLC (petroleum ether/EtOAc, 5:1): $R_f$ = 0.31.

***tert*-Butyl [(4-nitrophenoxy)carbonyl]-L-alaninate (21b):** General procedure **GP1** with L-alanine *tert*-butyl ester hydrochloride (114 mg, 0.630 mmol), DIPEA (0.11 mL, 81 mg, 0.63 mmol) and *p*-nitrophenyl chloroformate (158 mg, 0.783 mmol) in $CH_2Cl_2$ (5 mL). The reaction mixture was stirred for 23 h. Column chromatography ($CH_2Cl_2$ → $CH_2Cl_2$/MeOH, 98:2) gave **21b** as a colourless solid (112 mg, 57 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 1.47 (d, $J$ = 7.1 Hz, 3H, 3-H), 1.49 (s, 9H, OC($CH_3$)$_3$), 4.30 (dq, $J$ = 7.4, 7.1 Hz, 1H, 2-H), 5.77 (s, 1H, NH), 7.32 (d, $J$ = 8.9 Hz, 2H, 2′-H, 6′-H), 8.23 (d, $J$ = 8.9 Hz, 2H, 3′-H, 5′-H) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 18.87 (C-3), 28.10 [OC($CH_3$)$_3$], 50.51 (C-2), 82.75 [O*C*($CH_3$)$_3$], 122.13 (C-2′, C-6′), 125.25 (C-3′, C-5′), 144.95 (C-4′), 152.45 (carbamate-C=O), 155.91 (C-1′), 171.76 (C-1) ppm. HRMS (ESI): calcd. for $C_{14}H_{19}N_2O_6$ [M + H]$^+$ 311.1238, found 311.1250. IR (ATR): $\tilde{\nu}$ = 3307, 2941, 1735, 1700, 1526, 1371, 1217, 1148, 1002, 865 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 276 nm. TLC ($CH_2Cl_2$/MeOH, 95:5): $R_f$ = 0.71.

**SPPS of Val-Lys-Ala peptide unit (26a): 26a** was prepared according to general procedure **GP2**. Loading was carried out with carboxylic acid **19** (38.0 mg, 89.3 µmol) in $CH_2Cl_2$ (3 mL), 2-chlorotrityl chloride resin (79.1 mg, 90.2 µmol) and DIPEA (0.05 mL, 0.04 g, 0.3 mmol) over 19 h. The Fmoc group was cleaved, and *N*-Fmoc-L-alanine (164 mg, 0.527 mmol) was coupled using HBTU (201 mg, 0.530 mmol) and DIPEA (0.18 mL, 0.14 g, 1.1 mmol) over 1 h and 16 h. The peptide was Fmoc-deprotected, and $N^{\alpha}$-Fmoc-$N^{\varepsilon}$-Boc-L-lysine (251 mg, 0.536 mmol) was coupled using HBTU (219 mg, 0.577 mmol) and DIPEA (0.18 mL, 0.14 g, 1.1 mmol) over 1.5 h and 17 h. The peptide was Fmoc-deprotected, the urea unit was formed with *tert*-butyl [(4-nitrophenoxy)carbonyl]-L-valinate **21a** (105 mg, 0.310 mmol) and DIPEA (0.06 mL, 0.05 mg, 0.4 mmol) and the peptide was cleaved from the resin according to general procedure **GP3**. The resultant crude product was purified by column chromatography ($CH_2Cl_2$/MeOH, 98:2 → 95:5 → 9:1) to give **26a** (mixture of 1,3-dioxolane diastereomers) as a colourless solid (41.7 mg, 67 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.87 (d, $J$ = 6.2 Hz, 2 × 3H, Val-4-H), 0.92 (d, $J$ = 5.8 Hz, 2 × 3H, Val-4-H), 1.35 (d, $J$ = 6.3 Hz, 2 × 3H, Ala-3-H), 1.43 (s, 2 × 9H, Boc-OC($CH_3$)$_3$), 1.46 (s, 2 × 9H, OC($CH_3$)$_3$), 1.33–1.94 (m, 2 × 12H, Lys-3-H, Lys-4-H, Lys-5-H, 2-H, 3′-H, 4′-H), 2.11–2.17 (m, 2 × 1H, Val-3-H), 2.37–2.55 (m, 2 × 2H, 5′-H), 3.08–3.09 (m, 2 × 2H, Lys-6-H), 3.35–3.41 (m, 2 × 2H, 3-H), 3.54–3.56 (m, 2 × 1H, 1′-$H_a$), 3.88–3.92 (m, 2 × 1H, 2′-H), 4.04–4.06 (m, 2 × 1H, 1′-$H_b$), 4.26–4.32 (m, 2 × 2H, Val-2-H, Lys-2-H), 4.48–4.49 (m, 2 × 1H, Ala-2-H), 4.97–4.98 (m, 2 × 1H, 1-H) ppm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.14.

**SPPS of Val-Ala-Leu peptide unit (26b): 26b** was prepared according to general procedure **GP2**. Loading was carried out with carboxylic acid **19** (39.3 mg, 92.4 µmol) in $CH_2Cl_2$ (1.5 mL), 2-chlorotrityl chloride resin (96.3 mg, 110 µmol) and DIPEA (0.05 mL, 0.04 g, 0.3 mmol) over 18 h. The Fmoc group was cleaved, and *N*-Fmoc-L-leucine (196 mg, 0.555 mmol) was coupled using HBTU (210 mg, 0.554 mmol) and DIPEA (0.19 mL, 0.14 g, 1.1 mmol) over 2 h and 17 h. The peptide was Fmoc-deprotected, and *N*-Fmoc-L-alanine (196 mg, 0.630 mmol) was coupled using HBTU (207 mg, 0.546 mmol) and DIPEA (0.19 mL, 0.14 g, 1.1 mmol) over 2 h and 40 h. The peptide was Fmoc-deprotected, the urea unit was formed with *tert*-butyl [(4-nitrophenoxy)carbonyl]-L-valinate **21a** (95.0 mg, 0.281 mmol) and DIPEA (0.06 mL, 0.05 mg, 0.4 mmol) and the peptide was cleaved from the resin according to general procedure **GP3**.

The resultant crude product was purified by column chromatography ($CH_2Cl_2$/MeOH, 9:1) to give **26b** (mixture of 1,3-dioxolane diastereomers) as a colourless solid (28.6 mg, 53 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.83–0.91 (m, 2 × 12H, Val-4-H, Leu-5-H), 1.26 (d, $J$ = 6.6 Hz, 2 × 3H, Ala-3-H), 1.46 (s, 2 × 9H, $OC(CH_3)_3$), 1.50–1.92 (m, 2 × 9H, Leu-3-H, Leu-4-H, 2-H, 3′-H, 4′-H), 2.05–2.12 (m, 2 × 1H, Val-3-H), 2.33–2.45 (m, 2 × 2H, 5′-H), 3.31–3.42 (m, 2 × 2H, 3-H), 3.48–3.52 (m, 2 × 1H, 1′-$H_a$), 3.87–3.91 (m, 2 × 1H, 2′-H), 3.99–4.11 (m, 2 × 1H, 1′-$H_b$), 4.30–4.33 (m, 2 × 1H, Val-2-H), 4.42–4.49 (m, 2 × 2H, Leu-2-H, Ala-2-H), 4.93–5.00 (m, 2 × 1H, 1-H) ppm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.16.

**SPPS of Ala-Lys-Leu peptide unit (26c): 26c** was prepared according to general procedure **GP2**. Loading was carried out with carboxylic acid **19** (39.5 mg, 92.8 µmol) in $CH_2Cl_2$ (1 mL), 2-chlorotrityl chloride resin (86.0 mg, 98.0 µmol) and DIPEA (0.05 mL, 0.04 g, 0.3 mmol) over 19 h. The Fmoc group was cleaved, and *N*-Fmoc-L-leucine (212 mg, 0.600 mmol) was coupled using HBTU (221 mg, 0.583 mmol) and DIPEA (0.20 mL, 0.15 g, 1.2 mmol) over 2 h and 40 h. The peptide was Fmoc-deprotected, and $N^{\alpha}$-Fmoc-$N^{\varepsilon}$-Boc-L-lysine (280 mg, 0.598 mmol) was coupled using HBTU (240 mg, 0.633 mmol) and DIPEA (0.20 mL, 0.15 g, 1.2 mmol) over 2 h and 16 h. The peptide was Fmoc-deprotected, the urea unit was formed with *tert*-butyl [(4-nitrophenoxy)carbonyl]-L-alaninate **21b** (71.9 mg, 0.231 mmol) and DIPEA (0.07 mL, 0.05 mg, 0.4 mmol) and the peptide was cleaved from the resin according to general procedure **GP3**. The resultant crude product was purified by column chromatography ($CH_2Cl_2$/MeOH, 95:5 → 9:1) to give **26c** (mixture of 1,3-dioxolane diastereomers) as a colourless solid (31.8 mg, 48 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.85 (d, $J$ = 6.2 Hz, 2 × 3H, Leu-5-H), 0.89 (d, $J$ = 6.1 Hz, 2 × 3H, Leu-5-H), 1.31 (d, $J$ = 7.2 Hz, 2 × 3H, Ala-3-H), 1.41 (s, 2 × 9H, Boc-$OC(CH_3)_3$), 1.44 (s, 2 × 9H, $OC(CH_3)_3$), 1.27–1.33 (m, 2 × 2H, Lys-4-H), 1.41–1.93 (m, 2 × 13H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H, 2-H, 3′-H, 4′-H), 2.32–2.43 (m, 2 × 2H, 5′-H), 3.01–3.04 (m, 2 × 2H, Lys-6-H), 3.36–3.41 (m, 2 × 2H, 3-H), 3.47–3.52 (m, 2 × 1H, 1′-$H_a$), 3.86–3.90 (m, 2 × 1H, 2′-H), 3.97–4.11 (m, 2 × 1H, 1′-$H_b$), 4.31–4.37 (m, 2 × 1H, Ala-2-H), 4.42–4.47 (m, 2 × 1H, Lys-2-H), 4.47–4.53 (m, 2 × 1H, Leu-2-H), 4.92–5.01 (m, 2 × 1H, 1-H), 5.19–5.23 (m, 2 × 1H, Boc-NH), 6.18 (s, 2 × 1H, Ala-NH), 6.53 (s, 2 × 1H, Lys-NH), 7.40–7.53 (m, 2 × 1H, 3-NH), 7.64–7.68 (m, 2 × 1H, Leu-NH) ppm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.16.

**SPPS of Val-Lys-Leu peptide unit (26d): 26d** was prepared according to general procedure **GP2**. Loading was carried out with carboxylic acid **19** (36.8 mg, 86.5 µmol) in $CH_2Cl_2$ (1.5 mL), 2-chlorotrityl chloride resin (95.4 mg, 109 µmol) and DIPEA (0.05 mL, 0.04 g, 0.3 mmol) over 18 h. The Fmoc group was cleaved, and *N*-Fmoc-L-leucine (187 mg, 0.529 mmol) was coupled using HBTU (198 mg, 0.522 mmol) and DIPEA (0.18 mL, 0.14 g, 1.1 mmol) over 2 h and 17 h. The peptide was Fmoc-deprotected, and $N^{\alpha}$-Fmoc-$N^{\varepsilon}$-Boc-L-lysine (248 mg, 0.529 mmol) was coupled using HBTU (198 mg, 0.522 mmol) and DIPEA (0.18 mL, 0.14 mg, 1.1 mmol) over 2 h and 40 h. The peptide was Fmoc-deprotected, the urea unit was formed with *tert*-butyl [(4-nitrophenoxy)carbonyl]-L-valinate **21a** (70.0 mg, 0.207 mmol) and DIPEA (0.06 mL, 0.05 mg, 0.4 mmol) and the peptide was cleaved from the resin according to general procedure **GP3**. The resultant crude product was purified by column chromatography ($CH_2Cl_2$/MeOH, 9:1) to give **26d** (mixture of 1,3-dioxolane diastereomers) as a colourless solid (46.9 mg, 73 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.82–0.89 (m, 2 × 12H, Val-4-H, Leu-5-H), 1.43 (s, 2 × 9H, Boc-$OC(CH_3)_3$), 1.45 (s, 2 × 9H, $OC(CH_3)_3$), 1.25–1.90 (m, 2 × 15H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-4-H, Lys-5-H, 2-H, 3′-H, 4′-H), 2.07–2.10 (m, 2 × 1H, Val-3-H), 2.33–2.42 (m, 2 × 2H, 5′-H), 3.02–3.06 (m, 2 × 2H, Lys-6-H), 3.34–3.41 (m, 2 × 2H, 3-H), 3.51–3.54 (m, 2 × 1H, 1′-$H_a$), 3.87–3.92 (m, 2 × 1H, 2′-H), 4.01–4.13 (m, 2 × 1H, 1′-$H_b$), 4.28–4.34 (m, 2 × 2H, Val-2-H, Lys-2-H), 4.40–4.44 (m, 2 × 1H, Leu-2-H), 4.95–5.02 (m, 2 × 1H, 1-H) ppm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.22.

**Val-Lys-Ala peptide dithioacetal (27a):** General procedure **GP4** with 1,3-dioxolane **26a** (57.6 mg, 82.1 µmol), boron trifluoride diethyl etherate (1.50 µL, 1.73 mg, 12.2 µmol) and ethanethiol (0.12 mL, 0.10 g, 1.6 mmol) in $CH_2Cl_2$ (6 mL) over 5 d. After 4 d, additional boron trifluoride diethyl etherate (0.50 µL, 0.58 mg, 4.1 µmol) was added. DIPEA (0.08 mL, 0.06 g, 0.5 mmol) was then added. Column chromatography ($CH_2Cl_2$/MeOH, 95:5), gave **27a** as a colourless solid (40.0 mg, 72 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.87 (d, $J$ = 6.8 Hz, 3H, Val-4-H), 0.91 (d, $J$ = 6.8 Hz, 3H, Val-4-H), 1.22 (dt, $J$ = 7.4, 0.9 Hz, 6H, 2′-H), 1.33 (d, $J$ = 7.1 Hz, 3H, Ala-3-H), 1.30–1.37 (m, 2H, Lys-4-H), 1.42 (s, 9H, Boc-$OC(CH_3)_3$), 1.46 (s, 9H, $OC(CH_3)_3$), 1.38–1.49 (m, 2H, Lys-5-H), 1.55–1.64 (m, 1H, Lys-3-$H_a$), 1.68–1.75 (m, 1H, Lys-3-$H_b$), 1.98–2.03 (m, 2H, 2-H), 2.06–2.12 (m, 1H, Val-3-H), 2.53–2.60 (m, 2H, 1′-$H_a$), 2.62–2.69 (m, 2H, 1′-$H_b$), 3.04–3.08 (m, 2H, Lys-6-H), 3.36–3.42 (m, 1H, 3-$H_a$), 3.47–3.54 (m, 1H, 3-$H_b$), 3.82 (t, $J$ = 7.2 Hz, 1H, 1-H), 4.30–4.33 (m, 1H, Val-2-H), 4.40–4.41 (m, 1H, Lys-2-H), 4.58–4.64 (m, 1H, Ala-2-H), 5.07 (s, 1H, Boc-NH), 6.08 (d, $J$ = 7.9 Hz, 1H, Val-NH), 6.58 (s, 1H, Lys-NH), 7.32 (s, 1H, Ala-NH), 7.49 (s, 1H, 3-NH) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 14.56 (C-2′), 14.58 (C-2′), 17.91 (Val-C-4), 18.62 (Ala-C-3), 19.19 (Val-C-4), 22.71 (Lys-C-4), 24.13 (C-1′), 24.34 (C-1′), 28.26 [$OC(CH_3)_3$], 28.63 (Boc-$OC(CH_3)_3$), 29.78 (Lys-C-5), 31.84 (Val-C-3), 33.12 (Lys-C-3), 35.67 (C-2), 37.90 (C-3), 40.13 (Lys-C-6), 48.95 (C-1, Ala-C-2), 53.98 (Lys-C-2), 58.43 (Val-C-2), 79.15 (Boc-$OC(CH_3)_3$), 81.75 [$OC(CH_3)_3$], 156.48 (Boc-C=O), 158.26 (urea-C=O), 172.64 (Ala-C-1), 172.68 (Lys-C-1), 173.21 (Val-C-1) ppm. HRMS (ESI): calcd. for $C_{31}H_{60}N_5O_7S_2$ $[M + H]^+$ 678.3929, found 678.3932. IR (ATR): $\tilde{\nu}$ = 3281, 2970, 2930, 1725, 1629, 1542, 1366, 1161 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 224 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.46.

**Val-Ala-Leu peptide dithioacetal (27b):** General procedure **GP4** with 1,3-dioxolane **26b** (28.6 mg, 48.7 µmol), boron trifluoride diethyl etherate (0.60 µL, 0.69 mg, 4.9 µmol) and ethanethiol (60 µL, 50 mg, 0.81 mmol) in $CH_2Cl_2$ (3 mL) over 5 d. After 2 d, additional boron trifluoride diethyl etherate (0.30 µL, 0.35 mg, 2.5 µmol) was added. DIPEA (50 µL, 38 mg, 0.29 mmol) was then added. Column chromatography ($CH_2Cl_2$/MeOH, 98:2) gave **27b** as a colourless solid (23.4 mg, 85 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.86–0.93 (m, 12H, Val-4-H, Leu-5-H), 1.21 (t, $J$ = 7.4 Hz, 3H, 2′-H), 1.22 (t, $J$ = 7.4 Hz, 3H, 2′-H), 1.28 (d, $J$ = 7.0 Hz, 3H, Ala-3-H), 1.47 (s, 9H, $OC(CH_3)_3$), 1.52–1.71 (m, 3H, Leu-3-H, Leu-4-H), 1.96–2.04 (m, 2H, 2-H), 2.04–2.10 (m, 1H, Val-3-H), 2.55 (q, $J$ = 7.4 Hz, 1H, 1′-$H_a$), 2.58 (q, $J$ = 7.4 Hz, 1H, 1′-$H_a$), 2.61–2.69 (m, 2H, 1′-$H_b$), 3.39–3.52 (m, 2H, 3-H), 3.80 (t, $J$ = 7.2 Hz, 1H, 1-H), 4.30 (dd, $J$ = 8.8, 5.1 Hz, 1H, Val-2-H), 4.53–4.57 (m, 1H, Leu-2-H), 4.60–4.66 (m, 1H, Ala-2-H), 6.26 (d, $J$ = 8.8 Hz, 1H, Val-NH), 6.62 (d, $J$ = 6.5 Hz, 1H, Ala-NH), 7.64 (s, 1H, Leu-NH), 7.82 (s, 1H, 3-NH) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 14.57 (C-2′), 14.61 (C-2′), 18.09 (Val-C-4), 19.21 (Val-C-4), 19.89 (Ala-C-3), 22.53 (Leu-C-5), 22.87 (Leu-C-5), 24.16 (C-1′), 24.38 (C-1′), 24.98 (Leu-C-4), 28.29 [$OC(CH_3)_3$], 31.94 (Val-C-3), 35.74 (C-2), 37.97 (C-3), 41.42 (Leu-C-3), 49.05 (C-1), 49.37 (Ala-C-2), 52.06 (Leu-C-2), 58.51 (Val-C-2), 81.60 [$OC(CH_3)_3$], 157.93 (urea-C=O), 172.63 (Leu-C-1), 172.80 (Val-C-1), 174.17 (Ala-C-1) ppm. HRMS (ESI): calcd. for $C_{26}H_{51}N_4O_5S_2$ $[M + H]^+$ 563.3295, found 563.3293. IR (ATR): $\tilde{\nu}$ = 3272, 2963, 1731, 1630, 1543, 1367, 1257, 1145, 712 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 220 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.49.

**Ala-Lys-Leu peptide dithioacetal (27c):** General procedure **GP4** with 1,3-dioxolane **26c** (31.8 mg, 44.4 µmol), boron trifluoride diethyl etherate (0.55 µL, 0.63 mg, 4.4 µmol) and ethanethiol (0.05 mL, 0.04 g, 0.7 mmol) in $CH_2Cl_2$ (4 mL) over 5 d. After 3 d, additional

boron trifluoride diethyl etherate (0.28 µL, 0.32 mg, 2.2 µmol) was added. DIPEA (45 µL, 34 mg, 0.26 mmol) was then added. Column chromatography ($CH_2Cl_2$/MeOH, 98:2) gave **27c** as a colourless solid (22.6 mg, 74 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.87 (d, $J$ = 6.4 Hz, 3H, Leu-5-H), 0.90 (d, $J$ = 6.4 Hz, 3H, Leu-5-H), 1.22 (t, $J$ = 7.5 Hz, 3H, 2′-H), 1.22 (t, $J$ = 7.4 Hz, 3H, 2′-H), 1.29–1.33 (m, 2H, Lys-4-H), 1.32 (d, $J$ = 7.1 Hz, 3H, Ala-3-H), 1.42 (s, 9H, Boc-OC($CH_3$)$_3$), 1.46 (s, 9H, OC($CH_3$)$_3$), 1.42–1.48 (m, 2H, Lys-5-H), 1.51–1.71 (m, 5H, Leu-3-H, Leu-4-H, Lys-3-H), 1.97–2.02 (m, 2H, 2-H), 2.53–2.60 (m, 2H, 1′-H$_a$), 2.64 (q, $J$ = 7.5 Hz, 1H, 1′-H$_b$), 2.67 (q, $J$ = 7.5 Hz, 1H, 1′-H$_b$), 3.03–3.07 (m, 2H, Lys-6-H), 3.36–3.43 (m, 1H, 3-H$_a$), 3.47–3.53 (m, 1H, 3-H$_b$), 3.82 (t, $J$ = 7.1 Hz, 1H, 1-H), 4.37–4.43 (m, 1H, Ala-2-H), 4.48–4.49 (m, 1H, Lys-2-H), 4.56–4.61 (m, 1H, Leu-2-H), 5.12 (s, 1H, Boc-NH), 6.17 (d, $J$ = 5.4 Hz, 1H, Ala-NH), 6.69 (s, 1H, Lys-NH), 7.39 (s, 1H, Leu-NH), 7.74 (s, 1H, 3-NH) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 14.55 (C-2′), 14.59 (C-2′), 19.62 (Ala-C-3), 22.44 (Leu-C-5), 22.65 (Lys-C-4), 22.90 (Leu-C-5), 24.00 (C-1′), 24.37 (C-1′), 25.01 (Leu-C-4), 28.20 [OC($CH_3$)$_3$], 28.63 (Boc-OC($CH_3$)$_3$), 29.80 (Lys-C-5), 33.25 (Lys-C-3), 35.69 (C-2), 37.92 (C-3), 40.14 (Lys-C-6), 41.21 (Leu-C-3), 49.01 (C-1), 49.41 (Ala-C-2), 51.87 (Leu-C-2), 53.70 (Lys-C-2), 79.10 (Boc-OC($CH_3$)$_3$), 81.68 [OC($CH_3$)$_3$], 156.50 (Boc-C=O), 157.68 (urea-C=O), 172.49 (Leu-C-1), 173.35 (Lys-C-1), 173.70 (Ala-C-1) ppm. HRMS (ESI): calcd. for $C_{32}H_{62}N_5O_7S_2$ [M + H]$^+$ 692.4085, found 692.4088. IR (ATR): $\tilde{\nu}$ = 3270, 2970, 2931, 1736, 1628, 1543, 1230, 1162 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 221 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.47.

**Val-Lys-Leu peptide dithioacetal (27d):** General procedure **GP4** with 1,3-dioxolane **26d** (46.9 mg, 63.0 µmol), boron trifluoride diethyl etherate (0.78 µL, 0.90 mg, 6.3 µmol) and ethanethiol (70 µL, 59 mg, 0.95 mmol) in $CH_2Cl_2$ (4 mL) over 7 d. After 2 d and 4 d, additional boron trifluoride diethyl etherate (2 × 0.39 µL, 0.45 mg, 3.2 µmol) was added. DIPEA (60 µL, 46 mg, 0.35 mmol) was then added. Column chromatography ($CH_2Cl_2$/MeOH, 98:2) gave **27d** as a colourless solid (30.4 mg, 67 %). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ = 0.85–0.91 (m, 12H, Val-4-H, Leu-5-H), 1.23 (t, $J$ = 7.4 Hz, 6H, 2′-H), 1.31–1.37 (m, 2H, Lys-4-H), 1.44 (s, 9H, Boc-OC($CH_3$)$_3$), 1.46 (s, 9H, OC($CH_3$)$_3$), 1.46–1.76 (m, 7H, Leu-3-H, Leu-4-H, Lys-3-H, Lys-5-H), 1.95–2.03 (m, 2H, 2-H), 2.05–2.12 (m, 1H, Val-3-H), 2.54–2.61 (m, 2H, 1′-H$_a$), 2.63–2.70 (m, 2H, 1′-H$_b$), 3.04–3.13 (m, 2H, Lys-6-H), 3.30–3.37 (m, 1H, 3-H$_a$), 3.48–3.55 (m, 1H, 3-H$_b$), 3.82 (t, $J$ = 7.2 Hz, 1H, 1-H), 4.26–4.29 (m, 1H, Lys-2-H), 4.31–4.34 (m, 1H, Val-2-H), 4.39–4.43 (m, 1H, Leu-2-H), 5.06 (s, 1H, Boc-NH), 5.91 (d, $J$ = 7.0 Hz, 1H, Val-NH), 6.35 (s, 1H, Lys-NH), 6.89 (s, 1H, Leu-NH), 7.42 (s, 1H, 3-NH) ppm. $^{13}$C NMR (126 MHz, $CDCl_3$): $\delta$ = 14.59 (C-2′), 14.60 (C-2′), 17.81 (Val-C-4), 19.18 (Val-C-4), 22.15 (Leu-C-5), 22.39 (Lys-C-4), 23.01 (Leu-C-5), 24.06 (C-1′), 24.40 (C-1′), 25.04 (Leu-C-4), 28.26 [OC($CH_3$)$_3$], 28.64 (Boc-OC($CH_3$)$_3$), 29.78 (Lys-C-5), 31.89 (Val-C-3), 32.16 (Lys-C-3), 35.62 (C-2), 37.91 (C-3), 39.77 (Lys-C-6), 40.87 (Leu-C-3), 49.03 (C-1), 52.19 (Leu-C-2), 54.31 (Lys-C-2), 58.37 (Val-C-2), 79.27 (Boc-OC($CH_3$)$_3$), 81.92 [OC($CH_3$)$_3$], 156.70 (Boc-C=O), 158.39 (urea-C=O), 172.28 (Leu-C-1), 172.59 (Lys-C-1), 173.41 (Val-C-1) ppm. HRMS (ESI): calcd. for $C_{34}H_{66}N_5O_7S_2$ [M + H]$^+$ 720.4398, found 720.4405. IR (ATR): $\tilde{\nu}$ = 3271, 2964, 2931, 1630, 1544, 1366, 1252, 1161 cm$^{-1}$. UV ($CHCl_3$): $\lambda_{max}$ = 220 nm. TLC ($CH_2Cl_2$/MeOH, 9:1): $R_f$ = 0.49.

**Muraymycin analogue with Val-Lys-Ala peptide unit (28a):** General procedure **GP6** with aldehyde **14a** (3.8 mg, 6.7 µmol) and nucleoside **13**[12b] (3.9 mg, 6.7 µmol) in THF (2 mL) After 19 h, Amberlyst-15$^{TM}$ (spatula tip) and sodium triacetoxyborohydride (3.5 mg, 17 µmol) were added, and the mixture was further stirred for 21 h. Column chromatography ($CH_2Cl_2$/MeOH, 95:5) gave the fully protected muraymycin analogue. This was dissolved in 80 % TFA in water (1.5 mL) and stirred at r.t. for 24 h. The mixture was diluted with water and the solvent was evaporated under reduced pressure. The resultant crude product was purified by semipreparative HPLC (method 1) and lyophilised to give **28a** (bis-TFA salt) as a fluffy colourless solid (2.4 mg, 39 % over 2 steps). $^1$H NMR (500 MHz, $D_2O$): $\delta$ = 0.85 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 0.89 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 1.31 (d, $J$ = 7.3 Hz, 3H, Ala-3-H), 1.38–1.44 (m, 2H, Lys-4-H), 1.60–1.67 (m, 3H, Lys-5-H, Lys-3-H$_a$), 1.71–1.78 (m, 1H, Lys-3-H$_b$), 1.83–1.89 (m, 2H, 2′′-H), 2.04–2.11 (m, 1H, Val-3-H), 2.16–2.22 (m, 1H, 5′-H$_a$), 2.33–2.38 (m, 1H, 5′-H$_b$), 2.95 (t, $J$ = 7.6 Hz, 2H, Lys-6-H), 3.02 (dd, $J$ = 7.6, 7.6 Hz, 2H, 1′′-H), 3.18–3.29 (m, 2H, 3′′-H), 3.74 (dd, $J$ = 6.5, 6.5 Hz, 1H, 6′-H), 3.97 (d, $J$ = 5.3 Hz, 1H, Val-2-H), 4.01–4.04 (m, 1H, 3′-H), 4.04–4.06 (m, 1H, Lys-2-H), 4.09–4.13 (m, 1H, 4′-H), 4.15 (d, $J$ = 7.3 Hz, 1H, Ala-2-H), 4.37 (dd, $J$ = 5.7, 3.7 Hz, 1H, 2′-H), 5.71 (d, $J$ = 3.7 Hz, 1H, 1′-H), 5.83 (d, $J$ = 8.1 Hz, 1H, 5-H), 7.62 (d, $J$ = 8.1 Hz, 1H, 6-H) ppm. $^{13}$C NMR (126 MHz, $D_2O$): $\delta$ = 16.28 (Ala-C-3), 16.82 (Val-C-4), 18.60 (Val-C-4), 21.89 (Lys-C-4), 25.60 (C-2′′), 26.12 (Lys-C-5), 30.12 (Val-C-3), 30.74 (Lys-C-3), 32.96 (C-5′), 35.80 (C-3′′), 39.11 (Lys-C-6), 44.10 (C-1′′), 49.88 (Ala-C-2), 53.91 (Lys-C-2), 59.62 (Val-C-2), 60.31 (C-6′), 72.55 (C-2′), 72.80 (C-3′), 80.04 (C-4′), 91.63 (C-1′), 102.04 (C-5), 116.23 (q, $^1J_{CF}$ = 292 Hz, $F_3CCOO$), 142.73 (C-6), 151.33 (C-2), 159.46 (urea-C=O), 162.99 (q, $^2J_{CF}$ = 35.5 Hz, $F_3CCOO$), 166.16 (C-4), 172.49 (C-7′), 175.28 (Ala-C-1), 175.34 (Lys-C-1), 178.01 (Val-C-1) ppm. $^{19}$F NMR (376 MHz, $D_2O$): $\delta$ = –75.60 (TFA-$CF_3$) ppm. HRMS (ESI): calcd. for $C_{29}H_{49}N_8O_{12}$ [M + H]$^+$ 701.3464, found 701.3470. IR (ATR): $\tilde{\nu}$ = 3306, 3062, 2967, 1672, 1559, 1428, 1201, 1133, 800, 722 cm$^{-1}$. UV ($H_2O$): $\lambda_{max}$ = 260 nm. HPLC (method 1): $t_R$ = 19.6 min.

**Muraymycin analogue with Val-Ala-Leu peptide unit (28b):** General procedure **GP6** with aldehyde **14b** (8.8 mg, 19 µmol) and nucleoside **13**[12b] (14 mg, 24 µmol) in THF (3 mL). After 18 h, Amberlyst-15$^{TM}$ (spatula tip) and sodium triacetoxyborohydride (8.5 mg, 40 µmol) were added, and the mixture was further stirred for 23 h. Column chromatography ($CH_2Cl_2$/MeOH, 98:2 → 95:5) gave the fully protected muraymycin analogue. This was dissolved in 80 % TFA in water (1.5 mL) and stirred at r.t. for 24 h. The mixture was diluted with water and the solvent was evaporated under reduced pressure. The resultant crude product was purified by semipreparative HPLC (method 2) and lyophilised to give **28b** (TFA salt) as a fluffy colourless solid (8.9 mg, 58 % over 2 steps). $^1$H NMR (500 MHz, $D_2O$): $\delta$ = 0.84 (d, $J$ = 6.2 Hz, 3H, Leu-5-H), 0.89 (d, $J$ = 6.2 Hz, 3H, Leu-5-H), 0.90 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 0.94 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 1.31 (d, $J$ = 7.2 Hz, 3H, Ala-3-H), 1.52–1.64 (m, 3H, Leu-3-H, Leu-4-H), 1.86–1.92 (m, 2H, 2′′-H), 2.07–2.16 (m, 1H, Val-3-H), 2.27–2.34 (m, 1H, 5′-H$_a$), 2.42–2.47 (m, 1H, 5′-H$_b$), 3.07 (t, $J$ = 7.7 Hz, 2H, 1′′-H), 3.15–3.24 (m, 1H, 3′′-H$_a$), 3.28–3.33 (m, 1H, 3′′-H$_b$), 4.01 (dd, $J$ = 6.3, 6.3 Hz, 1H, 6′-H), 4.05–4.10 (m, 1H, 3′-H), 4.06 (d, $J$ = 5.4 Hz, 1H, Val-2-H), 4.09 (d, $J$ = 7.2 Hz, 1H, Ala-2-H), 4.13–4.17 (m, 1H, 4′-H), 4.20–4.23 (m, 1H, Leu-2-H), 4.41 (dd, $J$ = 5.7, 3.8 Hz, 1H, 2′-H), 5.72 (d, $J$ = 3.8 Hz, 1H, 1′-H), 5.85 (d, $J$ = 8.1 Hz, 1H, 5-H), 7.62 (d, $J$ = 8.1 Hz, 1H, 6-H) ppm. $^{13}$C NMR (126 MHz, $D_2O$): $\delta$ = 16.95 (Ala-C-3), 17.06 (Val-C-4), 18.53 (Val-C-4), 20.71 (Leu-C-5), 22.19 (Leu-C-5), 24.45 (Leu-C-4), 25.67 (C-2′′), 30.03 (Val-C-3), 32.60 (C-5′), 36.00 (C-3′′), 39.51 (Leu-C-3), 44.27 (C-1′′), 50.40 (Ala-C-2), 52.65 (Leu-C-2), 58.91 (C-6′), 58.94 (Val-C-2), 72.66 (C-2′), 73.00 (C-3′), 79.79 (C-4′), 91.89 (C-1′), 102.27 (C-5), 142.88 (C-6), 151.47 (C-2), 159.45 (urea-C=O), 166.25 (C-4), 171.53 (C-7′), 175.15 (Leu-C-1), 175.73 (Ala-C-1, Val-C-1) ppm. $^{19}$F NMR (376 MHz, $D_2O$): $\delta$ = –75.60 (TFA-$CF_3$) ppm. HRMS (ESI): calcd. for $C_{29}H_{48}N_7O_{12}$ [M + H]$^+$ 686.3355, found 686.3355. IR (ATR): $\tilde{\nu}$ = 3306, 2963, 1655, 1560, 1466, 1199, 1135, 721, 552 cm$^{-1}$. UV ($H_2O$): $\lambda_{max}$ = 260 nm. HPLC (method 2): $t_R$ = 19.4 min.

**Muraymycin analogue with Ala-Lys-Leu peptide unit (28c):** General procedure **GP6** with aldehyde **14c** (6.6 mg, 11 µmol) and nucleoside **13**[12b] (10 mg, 17 µmol) in THF (3 mL). After 18 h, Amberlyst-15$^{TM}$ (spatula tip) and sodium triacetoxyborohydride

(4.8 mg, 23 µmol) were added, and the mixture was further stirred for 22 h. Column chromatography ($CH_2Cl_2$/MeOH, 98:2 → 95:5) gave the fully protected muraymycin analogue. This was dissolved in 80 % TFA in water (1.5 mL) and stirred at r.t. for 24 h. The mixture was diluted with water and the solvent was evaporated under reduced pressure. The resultant crude product was purified by semipreparative HPLC (method 2) and lyophilised to give **28c** (bis-TFA salt) as a fluffy colourless solid (4.9 mg, 46 % over 2 steps). $^1$H NMR (500 MHz, $D_2O$): $\delta$ = 0.87 (d, $J$ = 6.1 Hz, 3H, Leu-5-H), 0.93 (d, $J$ = 6.1 Hz, 3H, Leu-5-H), 1.40 (d, $J$ = 7.3 Hz, 3H, Ala-3-H), 1.42–1.49 (m, 2H, Lys-4-H), 1.55–1.73 (m, 6H, Leu-3-H, Leu-4-H, Lys-5-H, Lys-3-$H_a$), 1.76–1.83 (m, 1H, Lys-3-$H_b$), 1.89–1.95 (m, 2H, 2″-H), 2.27–2.34 (m, 1H, 5′-$H_a$), 2.44–2.48 (m, 1H, 5′-$H_b$), 3.00 (t, $J$ = 7.6 Hz, 2H, Lys-6-H), 3.06 (dd, $J$ = 7.9, 7.9 Hz, 2H, 1″-H), 3.23–3.35 (m, 2H, 3″-H), 3.96 (dd, $J$ = 6.4, 6.4 Hz, 1H, 6′-H), 4.08–4.13 (m, 2H, 3′-H, Lys-2-H), 4.16–4.22 (m, 2H, 4′-H, Ala-2-H), 4.20 (d, $J$ = 7.3 Hz, 1H, Ala-2-H), 4.25–4.28 (m, 1H, Leu-2-H), 4.44 (dd, $J$ = 5.6, 3.8 Hz, 1H, 2′-H), 5.76 (d, $J$ = 3.8 Hz, 1H, 1′-H), 5.89 (d, $J$ = 8.1 Hz, 1H, 5-H), 7.66 (d, $J$ = 8.1 Hz, 1H, 6-H) ppm. $^{13}$C NMR (126 MHz, $D_2O$): $\delta$ = 16.72 (Ala-C-3), 20.67 (Leu-C-5), 22.11 (Leu-C-5), 22.15 (Lys-C-4), 24.47 (Leu-C-4), 25.68 (C-2″), 26.31 (Lys-C-5), 30.81 (Lys-C-3), 32.76 (C-5′), 36.01 (C-3″), 39.29 (Lys-C-6), 39.48 (Leu-C-3), 44.31 (C-1″), 49.30 (Ala-C-2), 52.65 (Leu-C-2), 54.26 (Lys-C-2), 59.42 (C-6′), 72.66 (C-2′), 73.00 (C-3′), 79.96 (C-4′), 91.88 (C-1′), 102.28 (C-5), 115.26–117.57 (m, $F_3CCOO$), 142.91 (C-6), 151.49 (C-2), 159.27 (urea-C=O), 166.26 (C-4), 171.84 (C-7′), 175.01 (Leu-C-1), 175.57 (Lys-C-1), 177.98 (Ala-C-1) ppm. $^{19}$F NMR (376 MHz, $D_2O$): $\delta$ = –75.60 (TFA-$CF_3$) ppm. HRMS (ESI): calcd. for $C_{30}H_{51}N_8O_{12}$ [M + H]$^+$ 715.3621, found 715.3628. IR (ATR): $\tilde{\nu}$ = 3294, 2960, 1670, 1558, 1430, 1200, 1133, 800, 722 cm$^{-1}$. UV ($H_2O$): $\lambda_{max}$ = 261 nm. HPLC (method 2): $t_R$ = 28.3 min.

**Reference muraymycin analogue with Val-Lys-Leu peptide unit (28d):**[10i] General procedure **GP6** with aldehyde **14d** (8.5 mg, 14 µmol) and nucleoside **13**[12b] (10 mg, 17 µmol) in THF (3 mL). After 18 h, Amberlyst-15$^{TM}$ (spatula tip) and sodium triacetoxyborohydride (6.3 mg, 30 µmol) were added, and the mixture was further stirred for 20 h. Column chromatography ($CH_2Cl_2$/MeOH, 98:2 → 95:5) gave the fully protected muraymycin analogue. This was dissolved in 80 % TFA in water (2 mL) and stirred at r.t. for 23 h. The mixture was diluted with water and the solvent was evaporated under reduced pressure. The resultant crude product was purified by semipreparative HPLC (method 2) and lyophilised to give **28d** (bis-TFA salt) as a fluffy colourless solid (7.0 mg, 52 % over 2 steps). $^1$H NMR (500 MHz, $D_2O$): $\delta$ = 0.84 (d, $J$ = 6.1 Hz, 3H, Leu-5-H), 0.89 (d, $J$ = 6.1 Hz, 3H, Leu-5-H), 0.90 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 0.94 (d, $J$ = 6.9 Hz, 3H, Val-4-H), 1.36–1.47 (m, 2H, Lys-4-H), 1.50–1.70 (m, 6H, Leu-3-H, Leu-4-H, Lys-5-H, Lys-3-$H_a$), 1.73–1.80 (m, 1H, Lys-3-$H_b$), 1.86–1.92 (m, 2H, 2″-H), 2.10–2.17 (m, 1H, Val-3-H), 2.25–2.31 (m, 1H, 5′-$H_a$), 2.40–2.45 (m, 1H, 5′-$H_b$), 2.97 (t, $J$ = 7.7 Hz, 2H, Lys-6-H), 3.06 (dd, $J$ = 8.1, 7.3 Hz, 2H, 1″-H), 3.20–3.31 (m, 2H, 3″-H), 3.93 (dd, $J$ = 6.4, 6.4 Hz, 1H, 6′-H), 4.05–4.08 (m, 2H, 3′-H, Val-2-H), 4.09–4.11 (m, 1H, Lys-2-H), 4.12–4.16 (m, 1H, 4′-H), 4.21–4.24 (m, 1H, Leu-2-H), 4.41 (dd, $J$ = 5.9, 3.8 Hz, 1H, 2′-H), 5.73 (d, $J$ = 3.8 Hz, 1H, 1′-H), 5.85 (d, $J$ = 8.1 Hz, 1H, 5-H), 7.63 (d, $J$ = 8.1 Hz, 1H, 6-H) ppm. $^{13}$C NMR (126 MHz, $D_2O$): $\delta$ = 17.04 (Val-C-4), 18.56 (Val-C-4), 20.72 (Leu-C-5), 22.10 (Lys-C-4), 22.16 (Leu-C-5), 24.46 (Leu-C-4), 25.70 (C-2″), 26.32 (Lys-C-5), 30.02 (Val-C-3), 30.83 (Lys-C-3), 32.79 (C-5′), 36.02 (C-3″), 39.30 (Lys-C-6), 39.58 (Leu-C-3), 44.32 (C-1″), 52.64 (Leu-C-2), 54.14 (Lys-C-2), 58.94 (Val-C-2), 59.44 (C-6′), 72.66 (C-2′), 73.00 (C-3′), 79.97 (C-4′), 91.88 (C-1′), 102.29 (C-5), 142.90 (C-6), 151.48 (C-2), 159.57 (urea-C=O), 166.25 (C-4), 171.84 (C-7′), 174.97 (Leu-C-1), 175.47 (Lys-C-1), 176.78 (Val-C-1) ppm. $^{19}$F NMR (376 MHz, $D_2O$): $\delta$ = –75.59 (TFA-$CF_3$) ppm. MS (ESI): calcd. for $C_{32}H_{55}N_8O_{12}$ [M + H]$^+$ 743.39, found 743.39. HPLC (method 2): $t_R$ = 22.3 min.

**Overexpression of MraY from *S. aureus*:** The overexpression of MraY was performed as described before.[10h,10i,17d]

**Fluorescence-based MraY assay:** The MraY assay was performed as described before.[10h,10i,17d]

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